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REVIEW

Current knowledge on innate immune gene biomarkers in crustaceans: a mini-review

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Abstract An increasing number of studies have supported the notion that strengthening the immune response can reduce the incidence of disease outbreaks in farming crustaceans. Although immunostimulants have been discovered, the mechanisms underlying the innate immune response in crustaceans remain elusive. This research emphasizes the versatile immune response exhibited by crustaceans against bacteria, viruses, and fungi, characterized by the presence of Toll, immunodeficiency (IMD), Janus kinase-signal transducers (JAK), and activators of transcription (STAT) pathways, as well as specialized immune effectors such as antimicrobial peptides (AMPs). In addition, this review provides an overview of studies exploring the impact of natural product supplementation on immune responses and primer sequences. Notably, this study provides a summary of the effects of natural product supplementation on immune response and gene expression in crustaceans. The results provide new insights into molecular biomarkers that can be applied to enhance the understanding of disease management in crustaceans.

Keywords Crustacean . Gene biomarkers . IMD Pathway . Innate immunity . JAK Pathway . Toll Pathway

Introduction

The occurrence of diseases in crustaceans has emerged as a significant constraint to aquaculture production. More than 50 viruses (Valente and Wan 2021), fungal infections (Davies et al. 2020), and bacterial pathogens (Quinitio 2017) have been reported to affect a diverse range of marine taxa commonly found in decapods. Infected crustaceans hinder the development of their morphological and physiological features, whereas other crustaceans lead to widespread mortality (FAO 2020). The ecology of juvenile crabs is most prevalent among crustaceans (Lavilla-Pitogo et al. 2004). These challenges negatively affect marine production worldwide (Caro 2020).

To date, there has been no specific cure for these diseases. The use of antibiotics, vaccination, hormones, and vitamins in the feed of marine species is generally used to eradicate infections and improve survival and growth (Amatul-Samahah et al. 2020). Consequently, this approach has been widely criticized owing to its cost, spread of drug-resistant pathogens and concerns about environmental sustainability (Karunasagar 2014). To address this gap, alternative strategies to control these diseases are necessary.

Considerable interest has been focused on boosting the immunity of marine species using immunostimulants to improve the growth, survival, and protection of crustaceans against inoculated pathogens (Barman et al. 2013). Immunostimulants are chemical compounds, stressors, or actions that enhance the immune system by interacting directly with cells, serving as the first line of defense (Traifalgar 2017).

According to Scopus®, a comprehensive database of scientific literature published by the British-Dutch publisher Elsevier, there has been an increasing number of studies concerning the use of immunostimulants against bacterial, fungal, and parasitic diseases in crustaceans over the past 10 years. Published research on

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the use of immunostimulants to enhance crustacean immunity increased from 8% in 2011 to 16% in 2020, with a total of 493 publications. Among these peer-reviewed studies, a wide variety of marine-derived immunostimulants for crustaceans are generally suggested for use in aquafeeds due to their availability, environmental friendliness, and cost-effectiveness (Huang and Ren 2020; Srivastava and Pandey 2015). Despite positive assessments of the use of immunostimulants, reports are limited to the characterization and behavioral and morphological responses of samples with finite adaptive markers (Moruf and Adekoya 2018).The use of cellular and genetic biomarkers to efficiently monitor the expression of molecules associated with immune system activation would significantly contribute to the body of knowledge. This review provides a comprehensive summary of immunostimulants and nonspecific defense mechanisms through the Toll, immunodeficiency (IMD), Janus kinase-signal transducers (JAK), and activators of transcription (STAT) pathways. These pathways are essential for recognizing and responding to microbial infections, including bacterial and viral threats (Pinkaew 2024).

In total, 493 scientific publications available from 2011 to 2024 concerning genes related to immune responses in crustaceans were assessed. Bibliographic information was accessed using keywords, such as genes, immunity pathways, immune responses, and crustaceans. Most studies were conducted in Asia (86%), followed by North America (7%), Europe (4%), and South America (3%). Relatively few studies have been published in Australia (1%). China made a significant contribution (74%) to the total number of studies conducted on gene expression, followed by Thailand (6%) and Taiwan (4%), with a smaller number of studies in Korea, Malaysia, the UK, Sweden, Denmark, Canada, Mexico, the USA, Cuba, France, Chile, Brazil, and Australia. However, only 2% of these studies used natural products as dietary supplements to enhance the immune response of crustaceans.

The use of natural sources of dietary supplements to enhance the immune response is important because of their health benefits, sustainability, low risk of resistance, and cultural significance. These supplements, derived from sources such as plants (Zhou et al. 2022), algae (Perveen et al. 2021), and microbes (Angela et al. 2020), have been shown to modulate immune responses, leading to improved resistance to pathogens. The results of related studies on these supplements offer a sustainable approach that may be cheaper, less likely to lead to resistance, and can hold cultural significance (Gombart et al. 2020).

Immunostimulation of nonspecific defense mechanisms

The immune system comprises two types of responses: nonspecific/innate immunity and specific adaptive/acquired immunity (Semple et al. 2020). Crustacean immunity relies heavily on the (nonspecific) innate immune system as initial protection against threats (Zhang et al. 2019). The key characteristic of a nonspecific immune system is the absence of immunological memory. In contrast, the specific immune system can remember foreign substances upon subsequent exposure to the same antigens (Huang et al. 2020). To effectively control crustacean diseases, it is crucial to deepen our understanding of crustacean innate immunity and the associated pathways.

The nonspecific immune system serves as the primary defense mechanism against invading pathogens in invertebrates. In crustaceans, it not only provides fundamental protection, but also plays an instructive role in acquired immunity and homeostasis (Srivastava and Pandey 2015). The innate immune response is initiated by pathogen exposure. Innate immune effector cells emit an early signal to detect invading pathogens through highly specialized receptors, known as pattern recognition receptors (PRRs) (Kaur et al. 2019). PRRs include Toll-like receptors (TLR), lipopolysaccharides, lectins, and other proteins (Huang et al. 2020). Studies have identified an array of PRRs involved in the production of protein enzymes in some crustacean species.

Toll signaling pathway in crustacean immunity: defense against fungi, bacteria, and viruses

The Toll pathway primarily functions as a defense mechanism against fungi, gram-positive bacteria, and viruses (Deepika et al. 2020). Several Toll-like receptors (TLRs) have been identified in crustacean species, including crabs, shrimps, and copepods. Among these, TLRs in shrimp, such as *Litopenaeus vannamei, Fenneropenaeus chinensis, Penaeus monodon, Macrobrachium rosenbergii, Procambarus immune system,*

and *Marsupenaeus japonicus*, have been extensively studied (Nie et al. 2018). Toll signaling plays a vital role in both innate and adaptive immune responses (Kawasaki and Kawai 2014). In innate immunity, the activation of the Toll pathway triggers downstream functions of enzyme proteins that regulate the expression of different antimicrobial peptides (AMPs). Initially, Spätzle was produced as a pro-Spätzle (Firmal 2020), acting as a ligand for the Toll receptor and stimulating the assembly of the receptor–adaptor complex involving myeloid differentiation factor 88 (MyD88). MyD88, containing a 1419 bp ORF encoding 472 (Chen and Wang 2019), initiates Pellino, a highly conserved protein that interacts with Pelle. The simultaneous involvement of proteins in this pathway results in the cactus-dorsal pathway, which acts as a positive regulator of the innate immunity in crustaceans (Hagyegi et al. 2010).

Toll-like receptors are crucial transmembrane molecules that link the extracellular compartment and signaling cascades, leading to cellular activation (Vasselon et al. 2002). In contrast, negative regulators of Toll signaling prevent autoimmune and inflammatory diseases (Tanji 2010). Thus, TLR-mediated pathways are involved in numerous aspects of the immune response, even in the absence of infection.

IMD signaling pathway: regulator of antimicrobial peptides and its implications in innate immunity

The Drosophila IMD pathway plays a crucial role in the modulation of antimicrobial peptides (AMPs) (Tanji 2010) and serves as a key component of the innate immune response. This signaling pathway exhibits distinct mechanisms, particularly in response to gram-negative bacteria and peptidoglycans (PGN) of gram-positive bacteria (Figure 1). Activation of the IMD pathway involves TGF-β interaction with the kinase 1 (Tak1) / AK1-associated binding protein (TAB2) complex, leading to the phosphorylation of the IκB-kinase (IKK) complex.

The innate immunity landscape is profoundly influenced by the interaction of I kappa-β kinase (IKK)-related kinases, TANK Binding Kinase 1 (TBK1), and IKKi with CDC10/Ankyrin (ANK) of Notch-4/Int3, which activates NF-κB (Raafat et al. 2017). ANK facilitate the transport of intracellular pyrophosphate (PPI) to the extracellular milieu, thereby modulating pathogen-specific immune responses (Bauer et al. 2015).

Functional studies by Kawasaki and Kawai (2014) indicated that IMD, TAK1, and TAB1 significantly affect downstream pathway genes and AMPs, including IKKβ, Relish, ALF1-6, and Crustin. The expression levels of NF-κB cascade members, such as dorsal, relish, TAK1, TAB1, Ikkβ, and IKKε, along with an array of protein enzymes (ALF4 and Cru1 & 2), were significantly downregulated (Zhou et al. 2018). The latter is crucial for the regulation of target gene expression, whereas the nuclear factor κB (NF-κB) transcription factor Relish translocates to the nucleus to regulate AMPs transcription (Liu et al. 2021).

AMPs integral to the innate immune system demonstrate broad-spectrum antimicrobial activity against pathogenic infections (Liu et al. 2021). Myllymäki et al (2021) presented evidence of the involvement of the IMD signaling pathway in immune defense against bacterial infection in crab species. The expression patterns of the IMD signaling pathway and NF-κB genes, including SpIMD, SpIKKβ, SpIKKε, SpRelish, and SpDorsal, exhibited distinct patterns in response to *Vibro alginolyticus*, suggesting their role in the bacterial infection response (Myllymäki et al. 2021). Furthermore, the substantial role of the IMD pathway as a potential modulator of neurodegeneration is currently under investigation, contributing to the expansion of our knowledge in this context.

JAK/STAT signaling pathway: mediator of cellular processes in response to stress, inflammation and immunity

The JAK/STAT pathway serves as a critical mediator of various cellular processes in response to diverse forms of inflammatory stimuli (Myllymäki et al. 2021), stressors, and immune challenges, particularly those involving cytokine receptors (Seif et al. 2017). A study by Bauer et al. (2015) suggested that the initiation of the JAK pathway protects crabs against MCRV infection, significantly influencing the expression of antiviral genes. The downregulation of JAK-STAT is intricately regulated by assembled regulator proteins, including suppressor of cytokine signaling (SOCS), protein inhibitors of activated STATs a/b (PIAS), and Protein Tyrosine Phosphatases (PTPs), in response to cytokine receptor binding. Following cytokine

binding to its receptor, JAKs, with a 3300 bp ORF encoding 1099 aa (Thomas et al. 2015), initiate transphosphorylation of specific tyrosine residues, creating docking sites for cytoplasmic transcription factors known as STATs (Raafat et al. 2017). Subsequent phosphorylation of STATs leads to the dimerization of the nucleus. STAT dimerization upregulates the expression of antiviral genes and modulates the polarization of T helper cells (Seif et al. 2017). The Stat92E transcription factor, TFAP-1, and Dsp1 form a complex that binds to the promoters of Relish target genes. Complex formation involves the engagement of different proteins and the replacement of Relish, indicating the necessity of immune signaling pathways for the appropriate regulation of these genes (Myllymäki et al. 2021).

Recent studies have elucidated serine phosphorylation of STAT1, which upregulates AMPs transcription in the nucleus. Therefore, phosphorylation plays a crucial role in the regulation of numerous signaling pathways. This review aimed to provide insights into the multifaceted functions and regulatory mechanisms of the JAK/STAT pathway in cellular responses to stress, inflammation, and immunity.

Immune-related genes expression using marine-derived supplementation

The innate immunity of crustaceans is the primary defense mechanism within a sophisticated regulatory network governed by a diverse set of genes. Pattern recognition receptors (PRR), in synergy with pathogen-associated molecular patterns (PAMP), elicit a cascade of immune responses involving genes such as LGBP, lectin, toll, B-actin, lysozyme, IMD, SOD, TLG 1, 2, and 3, STAT, NOX, CuZnSod, GADP, and proPO, thereby activating the immune system. These functions are initiated by the specific recognition of glycosylated PAMPs by crustacean proteins. Table 1 provides a comprehensive summary of published studies on biomarkers and supplementation with natural products, offering various extracts to crustaceans. Immune effectors induce transcription, thereby enhancing the overall immune response.

Yudiati et al. (2016) were the first to report the supplementation of *S. siliquosum* sodium alginate in the diet of *L. vannamei* at 2 g kg^{-1} feed, resulting in the significant upregulation of four actively related genes, namely LGBP, Toll, Lectin, and proPO. Notably, LPS-binding protein (LBP), β-1,3-glucan-binding protein (β-GBP), and lipopolysaccharide- and β-1,3-glucan-binding protein (LGBP) are involved in the activation of the proPO system and coagulation cascade and act as extracellular protein effectors, thereby increasing the rate of phagocytosis. In addition, the transcription levels of LGBP and Toll were the most prominent among *L. vannamei* in Indonesia.

A recent study Mansour et al. (2022) concluded that the administration of dietary *A. membranaceus* and *B. chinense* significantly influenced the expression of Lys, TLR, IMD, and mRNA in shrimp gills in China. This observation underscores the substantial impact of dietary supplementation on gene expression, thereby elucidating its potential for modulating immune responses in crustaceans. High expression of these genes, whether direct or indirect, reflected the upregulation of innate immunity, with no significant difference ($P > 0.05$) observed among the herbal treatments in terms of the growth and immune performance of shrimp. Thus, this study demonstrated that dietary supplementation with a single herb or a combination of these herbs contributed to an increase in the immune response of shrimp, ultimately leading to improved protection against bacterial infections. Studies in the Philippines have emphasized the impact of thermal immune responses and metabolic changes in crustaceans on their survival in complex aquatic environments (Shrestha et al. 2021).

In the United Kingdom, Hauton et al. (2004) presented the upregulation of immune-related gene sequences coding for proPO (AJ581662) and βGBP (AJ583519), which encode proteins of 683 and 367 amino acids with molecular weights of 78.1 and 41.7 kDa, respectively, and b-act (AJ581663) in the granulocytes isolated from *H. gammarus lobster*. Although significant, the contribution to immunity was modest, representing only a two-fold increase in the relative number of transcripts.

In a study conducted in India (Farook et al. 2015), upregulation of the expression of proPO, SOD, and lysozyme genes was observed in the post-larvae of the freshwater prawn, *M. rosenbergi*, exposed to an extra-small virus against white tail disease (WTD) using a plant extract derived from *C. dactylon* (scutch grass), as determined by real-time PCR analysis. However, the expression of crustin, peroxinectin, and anti-lipopolysaccharide immune genes was downregulated in MrNV- and XSV-infected larvae compared with that in healthy post-larvae.

Table 1 The biomarkers and supplementation on crustaceans

Supplementation	Primer sequence	GenBank accession no.	*Crustacean*	Finding/s	References
β-1,3-Glucan from *Euglena gracilis*	F: CAGGAGGGAATGTCCTTTGA R: GGTCCTTTCCACGTGTCTGT	FJ215871	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: TACGAAGGTGACACAGACAA R: TCTCTGAGAGGATGAGGATG	KR528473.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: CCTGGCTGGACCATCATTA R: CCGCTTCTTCCTTAGTGACT	1HM627757.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: TCTACCTGTCCTTCCTGCTC R: AGGTACAGGTGTTGGTCCAG	KU529651.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: GCCCAGTATGAGTCTACCTTC R: TCGTTCTTGTGAGTGTTACC		*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: GCAAGGACAACTACGATAAACACT R: GGAAAGTCACAAGCACCATCAT	FJ467931.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
β-1,3-Glucan from *Euglena gracilis*	F: GCTGCGATATTGACAAGCAA R: AGATGCAGTTCCCCGTACAC	FJ152103.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: TGAGGTGAAGGTAGAGGAT		*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	ACT CTA GGA AAC-3'	AJ581663	*Homarus gammarus*	UPREGULATED	Hauton et al. 2004
	F: CAGGAGGGAATGTCCTTTGA R: GGTCCTTTCCACGTGTCTGT	FJ215871	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: TACGAAGGTGACACAGACAA R: TCTCTGAGAGGATGAGGATG	KR528473.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F: CCTGGCTGGACCATCATTA	1HM627757.1	*Portunus trituberculatus*	UPREGULATED	Perveen et al. 2021
	F CATGTCCAACTTCGCTTTCAGA R ATCACCGCGTGGCATCTT	AF473579.2	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F TGGACCTAGCCACCATGCTT R GACCGATAGCCACCATGCTT	XM_027372426.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F GCCTTGGCAACGCTTTCA R CGCGCATCAGTTCAGTTTGT	EU284136.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F: GAGAGGCTGAACCGAGACTGA R: AAGAAAACGGCCCCCAATT	EU373096.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F:GCTAATCTGACCATTCCCTA R: TCTCGTCCAACTCGCTCT	DQ923424.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F: CATGCCTGCAGGACTGTTTA R: GGCCTGAGGGTAAGGTCTTC	JN180637.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F:TCGTACAACCAGCTGACGAG R: ATACTTCAGGTGGGCCACAG	JN180637.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F:CGGCTCTGCGGTTCACAT R: CCTCGACCTTGTCTCGTTCCT	FJ592176.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F:AGCCTGTCAGCGTGATGTTTT R: ACGGATGGCGAAGGCTTTA	KC779541.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	F: ATTCTGTGCGGCCTCTTTAC	F AY486426.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
Cacao pod husks hot-water extract	R: ATCGGTCGTTCTTCAGATGG F: GCTTTACTTCAATGGCAGGAT	MF135540.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	R: TGCGGGTGCGAGATCTG F: ATACCCAGGCCACCACCCTT	F DQ206403.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	R: TGACAGCAACGCCCTAACC F: CCACGAGACCACCTACAAC	F XM_027364954.1	*Litopenaeus vannamei*	UPREGULATED	Lee et al. 2020
	R AGCGAGGGCAGTGATTTC R: CCAGTGAAGTGAGCAGAG				
	F: CGG CAA CCA GTA CGG AGG ACC	Liva LGBP qPCR F	*Litopenaeus vannamei*	UPREGULATED	Yudiati et al. 2016
	R: GTG GAA ATC ATC GGC GAA GGA G	Liva LGBP qPCR R			
	F: TTC AAC GGT AGA CCC GTG ATTCTTC TCTT GCC GGG TTT AAG GTG AAC AGT	proPO-F proPO-R	*Litopenaeus vannamei*	UPREGULATED	Yudiati et al. 2017
	TTT GTA AAC AAC AGG CAG TTC CAC	Lectin V-F	*Litopenaeus vannamei*	UPREGULATED	Yudiati et al. 2018
Sargassum siliquosum with Calcium and Sodium Alginate	CTG TCT TTC ATC AGA ATG CTA CCTC	Lectin V-R			
	CAT GCC TGC AGG ACTG TTT A CCT CCA CCAT GAA GAT CAA GAT CAT	LvToll2-F (AF300705.2)	*Litopenaeus vannamei*	UPREGULATED UPREGULATED	Yudiati et al. 2019
	F: 5′ -CGACCTCGATCAGTACATGG-3 R: 5′ -GTAACCCTGGTGACAAGCCT-3′	AY170126.2	*Litopenaeus vannamei*	UPREGULATED	Angela et al. 2020
	F: 5′ -TGGTGCTTTCGTCAAACTTC-3′ R: 5′ -AACCTGGCCATACACAATGA-3′	DQ923424.1	*Litopenaeus vannamei*	UPREGULATED	Angela et al. 2021
40% crude *Astragalus membranaceus,* 7.5% crude lipid, Methionine	F: 5′ -ATCGAGGAACGAGACAAGGT-3′ R: 5′ -CGTACACTCGGTCGACATTC	FJ592176.1	*Litopenaeus vannamei*	UPREGULATED	Angela et al. 2022
	F: 5′-CGCGACCTCACAGACTACCT-3′ R: 5′-CTCGTAGGACTTCTCCAGCG-3′	FJ592176.1	*Litopenaeus vannamei*	UPREGULATED	Angela et al. 2023
	F: TGAAAGGGTCAGGTTGGG R:CCGCTCGTTTACATTAGA	EU077526.1	*Macrobrachium rosenbergii*	UPREGULATED	Farook et al. 2015
	F' GCCTCCAAAGAGGAAGAGTT R' CTCCAATTGCACCAACTTCC	DQ182596	*Macrobrachium rosenbergii*	UPREGULATED	Farook et al. 2016
	F' GCAGGTGACGGTTGAGGA R' ATGCGACTGACTGGTGGA	EF364558	*Macrobrachium rosenbergii*	DOWN REGULATED	Farook et al. 2017
	R' CACTGCTGCCTTCCGTTTC F' AGGGCTTGTGGATTATTCTG	JN572543	*Macrobrachium rosenbergii*	DOWN REGULATED	Farook et al. 2018
Cynodon dactylon Ethanol extract	AGTTGTGGCGGCTGCTGT CTGACGAAGTCTTGGGTT	FJ429306	*Macrobrachium rosenbergii*	DOWN REGULATED	Farook et al. 2019
	TGCTGGTTGGGCTTCTGG	AY257549.2	*Macrobrachium rosenbergii*	UPREGULATED	Farook et al. 2020

Table 1 Continued

	TTGTAGCGTTCGGTGTCG			UPREGULATED	
	F' CCCGACGGTCACTTGTTC	AY651918.1	*Macrobrachium rosenbergii*	UPREGULATED	Farook et al. 2021
	R' CGTGGATGCCGCAGGATT				
	5'-CAC ATT GAG GTA ACC AAC ACT GAA C3'	AJ581662	*Homarus gammarus*	UPREGULATED	Hauton et al. 2004
Listonella anguillarum granular haemocytes	5' -CTG CTC CAT GAA GGG CAT CT-3' 5'-AAC AGT GAC TAT GGC	AJ583519	*Homarus gammarus*	UPREGULATED	Hauton et al. 2004

Moreover, Lee et al. (2020), suggested that CPH (cacao pod husks) extract serves as a potential immune regulator of shrimp through parenteral administration, enhancing immunological responses against *V. alginolyticus* and tolerance against hypothermal stress in *L. vannamei.* The results revealed the upregulation of immune effectors such as LGBP, PE, proPO, proPO II, TLR2, IMD, ALF, and Pen3 in shrimp receiving CPH. On the other hand, upregulation of TLR1, TLR3, and STAT genes was accompanied by an increase in crustin and resistance to *V. alginolyticus* 1 d after shrimp received fresh CPH extract at 20 and 40 µg shrimp. This suggests that the CPH extract triggers the Toll and JAK/STAT signaling pathways to induce AMP production and increase resistance against bacterial and viral pathogens.

Buchmann (2014) explored the immunomodulatory potential of β-1,3-glucans from *E. gracilis* by elevating cellular enzyme responses to mRNA expression of genes associated with crab innate immunity. The mRNA transcription of seven immune-related genes, including proPO, TLR-2, ALF-1, NOX, Lysozyme, Crustin-1, and CuZnSOD, significantly enhanced the innate immune response against Mesanophyr spp. in a dose-dependent manner.

Most research findings indicate the involvement of proPO, Toll, LGBP, and crustin in the immunomodulation of various crustaceans. ProPO expression is consistently upregulated in the immune system of giant freshwater prawns, lobsters, and swimming crabs (Yudiati et al. 2016; Hauton et al. 2004; Farook et al. 2015; Buchmann 2014). This is attributed to the conversion of inactive proPO to PO (Yudiati et al. 2016), showing a positive correlation with the innate immune response of crustaceans against foreign microbes. The proPO system is a critical component of a cascade of proteolytic reactions that accompanies the recognition of invading organisms in crustaceans. Targeting these specific pathways and molecules is promising for controlling the increasing incidence of diseases in aquaculture.

Taken together, various studies have underscored the importance of sourcing dietary supplements to enhance immune responses from natural sources. These natural products not only possess immune-enhancing activities but also provide a range of benefits, such as regulating immune cell responses, improving resistance to pathogens, and supporting overall immune function. Exploring the impact of natural product supplementation on these genetic pathways further allows researchers to identify potential biomarkers that can be used to monitor and improve crustacean health. Additionally, these findings highlight the importance of understanding the genetic immune response, identifying key immune-related genes, shedding light on the molecular mechanisms, and offering a deeper understanding of how these organisms maintain health and cope with environmental stressors.

Overall, this study emphasizes the vital role of molecular studies in enhancing our understanding of crustacean immune defenses. The integration of natural product supplementation into these studies not only enhances the immediate health of crustaceans, but also provides a foundation for developing long-term strategies to protect these species from the growing threats posed by pathogens and environmental stressors.

Conclusions and perspectives

Amidst the rising demand for marine-derived species for drug discovery, reports on gene-related innate immune mechanisms are scarce. Strengthening the innate immune response in crustaceans not only fortifies their resistance to bacterial infections but also contributes to improved growth and survival. This strategic enhancement is intricately associated with various prophylactic measures, unraveling the complex pathways that activate specific molecules in the innate immune system.

Although extensive research has been conducted on the innate immunity of crustaceans, the activation of gene-related pathways has significantly advanced this field. A spectrum of PRRs, including Toll

receptors, LGBP, proPO, and crustin, along with specific immune effectors participating in the Toll, IMD, and JAK-STAT pathways, have emerged as potential targets for upregulating the immune system of crustaceans. Understanding these gene-immune specifications presents a successful approach to expand our knowledge regarding how these biomarkers respond to specific invading pathogens, countering their impact on the juvenile ecology of aquaculture.

Improving the environment of crustaceans involves bolstering their innate immune responses through research and the potential applications of the knowledge gained. By elucidating the mechanisms of innate immunity in crustaceans, such as the roles of specific receptors, such as Scavenger Receptor B2, in sensing pathogens and activating immune pathways (Buchmann 2014), researchers can develop strategies to enhance the immune defenses of crustaceans in their natural habitats. This review identified consistent gene patterns that could serve as a potential approach to establish cellular and molecular parameters. The provided evidence sheds light on the signaling pathways that activate these genes after exposure to threats, making these biomarkers potential targets for innovative therapeutic interventions with optimized small-molecule selectivity.

The development of dietary feed supplements to enhance the synthesis of immune proteins using a functional genomics approach holds promise for future advancement. These natural products not only possess immune-enhancing activities but also provide a range of benefits, such as regulating immune cell responses, improving resistance to pathogens, and supporting overall immune function. Therefore, the complexity and diversity of natural products provide opportunities for novel discoveries that can contribute to the advancement of both crustacean and human health.

To further deepen our understanding of future applications, it would be intriguing to explore considerations related to the physiology of crustaceans, including their metabolism and energy biosynthesis-associated metabolites, to further expand our understanding of their interactions with pathogens and to elucidate their modulation responses to diverse ligands.

Conflict of interest The authors declare that they have no conflicts of interest.

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