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Structural and functional diversity of lectins associated with immunity in the marine shrimp *Litopenaeus vannamei*

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ABSTRACT

Lectins are important pattern recognition receptors (PRRs) and their immunological action is related to the recognition of glycans present in the pathogen cells surface. The lectins described for *Litopenaeus vannamei* are divided into C-type, L-type and galectin, which are mainly expressed in hepatopancreas and hemocytes. They are involved in several immune response pathways, such as phagocytosis, hemocytes recruitment, prophenoloxidase activation, and gene regulation. Although lectins have multiple immune functions, most experimental challenges focus only on WSSV and *Vibrio* sp. This article is a detailed review on the role of lectins in *L. vannamei* immune system, bringing together information on molecular structure, temporal and special expression and immune function, highlighting the wide participation of these molecules in shrimp innate immune system.

1. Introduction

The immune response capacity of shrimps is mostly dependent on the innate immune system [1]. The activation of immunological cascades starts with the recognition of pathogens, mainly through the action of pattern recognition receptors (PRRs), such as lectins [2,3]. The PRRs identify and bind to components present on the surface of microorganisms, known as pathogen associated molecular patterns (PAMPs), inducing cellular and humoral responses [3,4]. Humoral responses in general involve the production of antimicrobial molecules (*e.g.* antimicrobial peptides, lysozyme and lectins) [5]. Cellular responses, on the other hand, are mainly represented by phagocytosis (engulfment and elimination of microorganisms by the hemocytes) and hemocyte recruitment (increased production and delivery of hemocytes to the sites of infection) [1,6–9].

Lectins are defined as proteins capable of recognizing and binding carbohydrates or glycoproteins in a specific way [10]. Due to this ability, several previous studies report the involvement of lectins in immune signaling pathways in crustaceans, participating in opsonization, phagocytosis, activation of the proPO cascade, and regulation of the expression of other immune genes, such as antimicrobial peptides (AMPs) [11–14]. However, the immune roles of *Litopenaeus vannamei* lectins are still poorly explored, requiring further studies on mechanisms of expression regulation, cell signaling pathways and activity against specific pathogens [15].

The marine shrimp *L. vannamei* accounts for more than 50% of all crustaceans produced and consumed worldwide, making it the main species in modern shrimp farming [16]. However, the productivity is usually hampered by the occurrence of disease outbreaks, mainly of bacterial and viral etiology, which are responsible for losses of millions of dollars per year [17,18]. Understanding the immune system of *L. vannamei* seems to be a necessary step to achieve new and more robust strategies to mitigate the effect of diseases and, consequently, to reduce losses to the activity [19]. This article is a compilation of specific information about the structural and functional characterization of immune lectins of the Pacific white shrimp *L. vannamei*.

2. Classification of L. *vannamei* lectins

Lectins are classified according to their structural characteristics and carbohydrate recognition domains (CRDs) [20]. The lectin-sugar specificity is determined by the CRD and its cofactors, which are involved in molecular conformational stability [10]. Structural and composition similarities between CRDs make it possible to classify animal lectins into 15 groups: C-type, galectins, P-type, I-type, pentraxins, heparin-binding type, F-type, calnexin/calreticulin, M-type, L-type, R-type, F-box, ficolins, chilectins and interlectins [21]. However, all shrimp lectins described to date fall into only seven of these families, which are C-type, L-type, P-type, M-type, galectins, fribrinogen-like domain lectins (subgroup of ficolins), and calnexin/calreticulin [22].

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At this moment, according the available references, only three lectin types have been associated with *L. vannamei* immunology functions. As previously observed for many invertebrates, the majority of the lectins described for *L. vannamei* are C-type lectins, whose binding activity is Ca^{2+}-dependent [23]. The second type is galectin, which specifically recognizes β-galactoside. The third type is L-type lectin, whose binding domain shows high homology to the leguminous lectins domain, which is able to recognize several carbohydrates [10,24,25]. The diversity of lectin groups and forms suggest multiple functions in *L. vannamei* immune system (Table 1).

Previous reviews indicate that lectins perform a wide number of roles connected to the innate immunity of shrimp, due to the diversity of reported domains and activity sites [14,26]. Shrimp lectins can be found inside and outside cells, acting mainly in molecular trafficking, cell-cell interaction, and signaling [12]. Most of the investigated lectins are C-type lectins, highlighting the contribution of these molecules to the immunity of these animals.

3. Structural diversity of *L. vannamei* lectins

Most of the knowledge about animal lectins is based on studies with vertebrates. However, they are similar to invertebrate lectins in terms of structural characteristics. Structurally, most C-type lectins are characterized by the presence of a single CRD per subunit [14,27]. In general, the size of CRDs are approximately 120–130 amino acids, organized in two α-helix, two β-sheets and inter loops, stabilized by means of disulfide bridges established between cysteine residues [27,28]. Most CRDs are held by four cysteines (Fig. 1), which form two bridges [29]. The maintenance of CRD preserves the conformation of the protein quaternary structure and its biological functions [30,31].

The L-type lectins, in turn, are distinguished from other lectins mainly by their tertiary structure, called “jelly-roll fold” [32]. This structure is characterized by the usual α-helix in its CRD, and by flat and concave antiparallel β-sheets connected by a short sheet, with intercalated loops [10]. In general, its activity is dependent on metallic ions such as Ca^{2+} or Mg^{2+}. However, in the LvLTLC1 case, its activity is not dependent, but rather intensified in the presence of Ca^{2+} [33]. On the other hand, the galectins exhibit a CRD of approximately 130 amino acids, with well conserved residues responsible for recognizing β-galactosides and no ionic dependency [25,34]. The three-dimensional structure of galectins is based on anti-parallel β-sheets and an α-helix, differing from L-type lectins mainly by the organization in β-sandwiches [35].

Although most lectins have a single CRD, lectins with two CRDs have already been reported for *L. vannamei*. Low-density lipoprotein receptor class A (LDLa) and transmembrane region (TM) have been identified as well (Fig. 2, Table 2). The presence of two CRDs or additional domains provides structural diversity, expanding the spectrum of intermolecular interactions and may act in functional synergism [36,37]. Most lectins also have a signal peptide (SP) in their structure, suggesting that these proteins are carried to the extracellular environment in the post-translational process, where they are transported by the hemolymph and exercise their immunological functions as circulation humoral molecules [38,39]. Other lectins, which do not have SP, remain indefinitely in the cell after their translation, acting in cell signaling and traffic pathways [40]. The functional annotation of LvGal (http://eggnog-mapper.embl.de/) indicates that this lectin participates in extracellular structures [41].

Regarding specific carbohydrate binding sites, there are two well-conserved amino acid motifs among C-type lectins: EPN (Glu-Pro-Asn), responsible for binding to mannose; and QPD (Gln-Pro-Asp), responsible for binding to galactose [29]. However, these motifs are often mutated into other similar motifs, maintaining their functionality and specificity, such as EPD (LvCTL3), EPA (LvCTL4 and LvCTL4.2), QPG (LvCLT5) and QAP (LvCTLD) [43–47]. Another common amino acid motif, which is also of structural importance, is the WND (Trp-Asn-Asp). However, this motif has not been identified in any *L. vannamei* lectin, being replaced by its mutants motifs, such as WYD (LvCTL1), WNH (LvPLP) and MND (LvLT) [11,48,49] (Table 2). The binding motifs play an important role in the final stability of the protein, interacting with carbohydrates and Ca^{2+} ions, showing that lectins with different affinities act over a wide recognition spectrum [50].

4. Phylogenetic analysis of *L. vannamei* lectins

The phylogenetic tree constructed using the amino acid sequences of lectins described for *L. vannamei* highlighted that these proteins can be grouped into two broader clusters, the first of them grouping all C-type lectins (Fig. 3). As expected according to the literature, the second cluster was formed by the L-type lectin and galectin, as these two lectin structures are known to be more related to each other than to the C-type lectins [21,51].

Within the C-type lectin group, there was a clear distinction between proteins with a single CRD and proteins with a more complex organizational structure, such as two CRDs (LvLT and LvCTL-br) and LDLa (LvCTLD and LvLdlrCTL). These data reiterate that, even though there is a large number of lectins (especially C-type lectins) these proteins have very similar domain structures. The number of lectins, the maintenance of the molecular architecture and the evolutionary divergence between the groups also indicate the immunological importance of these molecules during the evolution and adaptation of *L. vannamei* immunological system [31,52].

When building a phylogenetic tree using only the sequences of the CRDs of *L. vannamei* lectins, it was observed that the division into two large groups (C-type lectins and L-type lectin/galectin) still allows, highlighting the structural similarity between partial or complete

Table 1
Summary of Litopenaeus vannamei lectins.

Lectin groups	Domains	Tissue distribution	Reported functions	Gene ID	Gene symbol
C-type lectins	C-type lectin domain (CTLD)	Brain, epithelium, gills, gonad, heart, hemocytes, hepatopancreas, intestine, muscle, nerves, pyloric caecum and stomach	Pattern recognition, proPO activation, phagocytosis induction, melanization, encapsulation, cellular signaling, and gene regulation	113800422; 113803845; 113805243; 113805525; 113805762; 113809992; 113812325; 113816216; 113818670; 113820005; 113827432	LOC113800422; LOC113803845; LOC113805243; LOC113805525; LOC113805762; LOC113809992; LOC113812325; LOC113816216; LOC113818670; LOC113820005; LOC113827432
L-type lectins	L-type lectin domain (LTLD)	Gills and hepatopancreas	Pattern recognition and bacterial clearance	113829606	LOC113829606
Galectins	Galectin type domain (GLECT)	Gills, hemocytes and hepatopancreas	Pattern recognition, bacterial clearance and gene regulation	113805092	LOC113805092

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CRD-LvLec1 (not deposited)    1    CPDTFFEA-----GGG-CFHVLDTGDTDITWEDARETCIGLSDSGWTVDLASLDSTAQLE        54
CRD-LvCTLU (QGW58119)         1    CPDTFFEA-----GGG-CFHVVDTGDTDITWEDARETCIGLSDSSWTVDLASLDSTAQLE        54
CRD-LvPLP (XP_027210459)      1    CPPNFIRL-----GNS-CYYFST---DMNSWHSAHFACRALNS-----QLAALET---LW        43
CRD-LvLectin-1 (ADW08726)     1    CPTGYVDFWLDSVTPV-CLSFATY--GKGTWTNLRQMCQAQGA-----DLAKLDG----N        48
CRD-LvLectin-2 (ADW08727)     1    CPVGWVDFSVDPMSPV-CLKFVMY--GKGTWYSLRGLCQAEGA-----DLAELRG----E        48
CRD-LvCTLD (AEH05998)         1    CPRFYTRV-----GGQ-CLSVFYV--GSSSWGEARSFCKHIGG-----DLLSIQN---VN        44
CRD-LvLdlrCTL (AYA22372)      1    CPHLYTRV-----GDH-CLSIFFF--GLVNWGEARAFCKMNGG-----DLLSFQDG--FE        45
CRD-LvCTL4 (AKA64754)         1    CSDGWLDI-----NGV-CFYFHQD--QSMPWEDAKRFCETSGA-----VLAKVAN---AQ        44
CRD-LvLec (ABU62825)          1    CPYPYEPL-----DDTRRIFLDAY--VTYTWQDTVDPCKTHSG-----EILMIED---CE        45
CRD-LvCTL1 (DQ858900)         1    CPYPYEPL-----DDTRCIFLDAY--VTCTWQDTVDLCKTHSG-----EILMIED---CE        45
CRD-LvCTL4.2 (QZL13787)       1    CPGNFLNF-----GET-CLYLAKD--IGITWEAALLFCQDLGG-----HLAVFRD---AN        44
CRD1-LvLT (ABI97374)          1    CPGGYSLV-----GDK-CLLFVTF--VAEPYGEARQFCHAAKG-----ELAAITT---AA        44
CRD2-LvLT (ABI97374)          1    CPVLFIEI-----GGL-CLMFVTW--AEETWEDARRACAGASA-----ELLAITD---VE        44
CRD1-LvCTL-br (ADU25463)      1    CPGGYSLV-----GDK-CLLFVTF--VAEPYGEARQFCHAAKA-----ELAAITT---AT        44
CRD2-LvCTL-br (ADU25463)      1    CPVLFIEV-----GGL-CLMFVTW--AEETWEDARRACTGASA-----ELLAITD---VE        44
CRD-LvCTL3 (AGV68681)         1    CDGGFHNI-----YDH-CIQFRT---QEVSWYEGKNLCSNMGA-----KLAKVDD---AN        43
CRD-LvAV (AGC54451)           1    CYSPYTPI-----GNR-CLFVEPQ--TEGTWYQMRDFCYLVNG-----NLLKLDD---AN        44
CRD-LvCTL5 (QGA67284)         1    CPHPFVDI-----NGR-CLFINNF--AQMTWEAARGFCQGFQA-----DLVAVDE---AN        44
                                   *                                 *

CRD-LvLec1 (not deposited)  111  VYIEDVTMTSGSES--------------------------RGRFYASCTMTDALQRALCR      144
CRD-LvCTLU (QGW58119)       111  VFIEDVTMTSGSES--------------------------RGRFYASCTMTDALQRALCR      144
CRD-LvPLP (XP_027210459)     91  ----------------------TPQFP-RSSRWHCLFLSPVINYRWNHDLCTQFMHYLCE      127
CRD-LvLectin-1 (ADW08726)   101  ---------------------------QPNHGTAANYACLYAPDFF-FHSCDNDRKIYAICQ      134
CRD-LvLectin-2 (ADW08727)   101  ----------------------HPAQPDGGTSSNYACIYTPDFY-FHSCKNDIKIYAICQ      137
CRD-LvCTLD (AEH05998)       101  ---RNVTLTGTSEVREANNGECYHYTQAPETPPKGFCAAITYDKHFYMSDEDCLADMSPLCV      159
CRD-LvLdlrCTL (AYA22372)    100  ---RNQTISL--------KKSCNRYLQRPRKPTVGMCTALNFENFFYMSDEDCLAKKSPLCV      150
CRD-LvCTL4 (AKA64754)       100  ----------------------WAHEPAGGADENCLLLDETRKYYFNDANCNLIHHPICM      137
CRD-LvLec (ABU62825)         97  --------------------------EPNSGNTHNCAIMHASYNHYWYDIQCENKYNPICL      131
CRD-LvCTL1 (DQ858900)        97  --------------------------EPNSGNTHNCAIMHASYNHYWYDIQCENKYNPICL      131
CRD-LvCTL4.2 (QZL13787)      98  ----------------------VREPAQGAAANCAFLHGPDDFLIHDAPCDWKVMPLCE      134
CRD1-LvLT (ABI97374)         99  ----------------------QPDNA--HENEHYLCLSSSWFLYMNDASSSAIINFICE      134
CRD2-LvLT (ABI97374)         99  ----------------------SAQEPDGGTNQNCLAITGEGYFNFRDYSCASKFNPLCV      136
CRD1-LvCTL-br (ADU25463)     99  ----------------------QPDNT--HGNEHCLFIPSWWFFYMADNPCSVVKNFICE      134
CRD2-LvCTL-br (ADU25463)     99  ----------------------SYPGTRWRNRSELPCDHWRRYFNFRDYSCASKFNPLCV      136
CRD-LvCTL3 (AGV68681)        96  ----------------------QVQEPDSNYNHNCVFLARHDHFFFFDYDCGAPEAIICE      133
CRD-LvAV (AGC54451)          97  ----------------------VAQQPNGGGSENCAVMRWDSFYHIHDESCYTVRSVICE      134
CRD-LvCTL5 (QGA67284)        96  ----------------------DYQQPGGQTNENCISLSKDNYFFFDDLDCNEEIAVICE      133
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Fig. 1. Alignment of carbohydrate recognition domain (CRDs) of L. vannamei C-type lectins. In grey, the cysteines responsible for disulfide bridges. The potential disulfide bonds were predicted using ScanProsite program (https://www.expasy.org/resources/scanprosite).

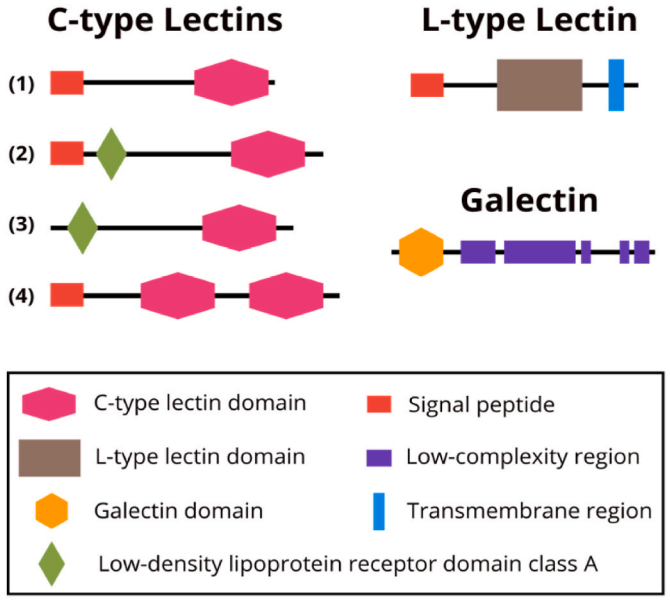

Fig. 2. Domains and sites architecture of L. vannamei lectins. The architecture of sequences were predicted by Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/) [42]. C-type lectins were classified in four regular architectures: (1) signal peptide and one CRD; (2) signal peptide, low-density lipoprotein receptor and one CRD; (3) no signal peptide, low-density lipoprotein receptor and one CRD; (4) signal peptide and two CRDs. L-type lectin has a signal peptide region, one CRD and a transmembrane region. Galectin presents one CRD and many low-complexity regions.

Table 2
Structural information from sequences of L. vannamei lectins.

Name	GenBank N°	CRD	Length of CRD	Ca^{2+}-binding site	Special feature
LvLT	ABI97374	2	134, 136	QPD, MND, EPD, FRD	SP
LvLec	ABU62825	1	131	EPN, WYD	SP
LvCTL1	DQ858900	1	131	EPN, WYD	SP
LvCTL-br	ADU25463	2	134, 136	QPD, MAD, F (L)RD	SP
LvLec1	–	1	144	EPA	SP
LvCTLD	AEH05998	1	159	QAP	LDLa
LvLectin-1	ADW08726	1	137	QPD	SP
LvLectin-2	ADW08727	1	134	QPN	SP
LvCTL3	AGV68681	1	133	EPD	SP
LvAV	AGC54451	1	134	QPN, WIG	SP
LvCTL4	AKA64754	1	137	EPA	SP
LvLdlrCTL	AYA22372	1	150	WIG	SP and LDLa
LvCTL5	QGA67284	1	133	QPG	SP
LvCTLU	QGW58119	1	144	WVG	SP
LvPLP	XP_027210459	1	127	WNH	SP
LvCTL 4.2	QZL13787	1	134	EPA	SP
LvLTLC1	ATP62320	1	226	–	SP and TM
LvGal	AGV04659	1	134	–	LCR

Abbreviations: SP, signal peptide; LDLa, low-density lipoprotein region class A; TM, transmembrane region; LCR, low-complexity region.

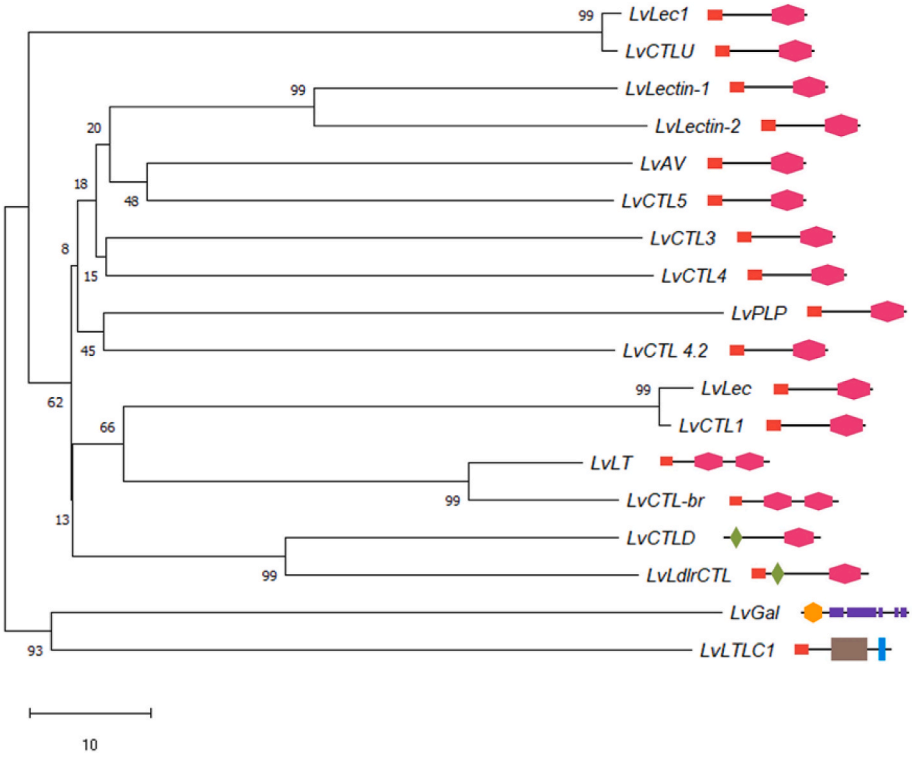

Fig. 3. Neighbor-Joining tree of the complete amino acid sequences of L. vannamei lectins. Indicated lectin architecture follows the schemes set in Fig. 2. The abbreviations and accession numbers of L. vannamei lectins are listed in Table 2. The tree was constructed using Molecular Evolutionary Genetics Analysis software (MEGA), version X [53]. Numbers near branches indicate bootstrap support after 10,000 replicates, the evolutionary distances were computed using the p-distance method and all positions containing gaps and missing data were completely eliminated [54].

sequences these molecules (data not shown). Furthermore, C-type lectins with architecture type 4 (LvLT and LvCTL-br, see Fig. 2) showed homology, respectively, between their CRD1 and CRD2, suggesting their strong structural similarity in the active sites.

5. Temporal and spatial expression of *L. vannamei* lectins

5.1. Tissue distribution

Current knowledge on the distribution of lectins in arthropod tissues comes mainly from studies with insects, where the majority of lectins are expressed in hemocytes and a lower number in the fat body (tissue

Table 3
Tissue distribution and functions of L. vannamei immunity lectins.

Name	Tissue distribution	Respond to challenge	Functions	Reference
LVL	Hemolymph	–	–	[61]
LvLT	Hepatopancreas	–	Antiviral activity	[48]
LvLec	Brain, hemocytes and hepatopancreas	–	Phagocytosis induction and PO liberation	[62,63]
LvCTL1	Hepatopancreas	WSSV	PRR and bind proteins WSSV	[49]
LvCTL-br	Gills	IHHNV	–	[59]
LvLec1	Hepatopancreas	WSSV	PRR	[64]
LvCTLD	Gils and nerves	YHV	Melanization and encapsulation induction, binding to YHV particles, proPO activation and hemocytes recruitment	[47]
LvLectin-1	Gonad, hepatopancreas and muscle	*V. anguillarum*	PRR	[60]
LvLectin-2	Gills, hemocytes, hepatopancreas and muscle	WSSV *V. anguillarum*	PRR	[60]
LvCTL3	Gills and hemocytes	WSSV *V. parahaemolyticus*	PRR and NF-kB signaling	[43]
LvAV	Hepatopancreas	WSSV	Anti-WSSV activity	[65]
LvCTL4	Gills, intestine, epithelium and hepatopancreas	*V. parahaemolyticus*	NF-kB signaling and antimicrobial response	[44]
LvLdlrCTL	Stomach, heart, gills and hemocytes	*V. parahaemolyticus*	Phagocytosis induction, and NF-kB and JAK-STAT signaling	[66]
LvCTL5	Hepatopancreas	WSSV *V. parahaemolyticus*	Phagocytosis induction, microbiostatic regulation and gene expression regulation	[46]
LvCTLU	Hepatopancreas, pyloric caecum and hemocytes	WSSV	Agglutination, phagocytosis induction and ER-stress response	[67]
LvPLP	Hepatopancreas, heart, gills, hemolymph and hemocytes	–	Bacterial binding and agglutination, gene expression regulation and phagocytosis induction	[11]
LvCTL 4.2	Stomach, nerve and lymphoid organ	*V. parahaemolyticus*	PRR	[45]
LvLTLC1	Gills and hepatopancreas	*V. harveyi*	PRR and induction of bacterial clearance	[33]
LvGal	Gills, hemocytes and hepatopancreas	*V. anguillarum* *V. alginolyticus*	PRR, induction of bacterial clearance, phagocytosis induction and gene expression regulation	[68,69]

analogous to the hepatopancreas present in crustaceans) [19,26,55,56]. Although most arthropod lectins are synthesized by hemocytes, the biological synthesis of lectins in crustaceans is divided almost equally between hemocytes and hepatopancreas; however, the majority of *L. vannamei* immunity lectins are synthesized in the hepatopancreas [14,26].

After the synthesis in the hepatopancreas, the most likely pathway is that the lectins are released to the hemolymph, captured by hemocytes and stored inside the granules [13,19,56,57]. During infectious processes, the lectins are recruited by the circulatory system and can act as humoral molecules, recognizing and signaling the presence of PAMPs [13,19,47]. Most of the *L. vannamei* lectin genes involved in infection response are specifically expressed or have their highest expression level in the hepatopancreas, such as LvLT, LvCTL1 and LvCTL5 [46,48,49] (Table 3).

Lectins synthesized by hemocytes are directly stored in the cytoplasmic granules and later released by degranulation, after an infection [13,19,58]. The high level of lectin expression in hemocytes indicates that, in addition to storing and releasing lectins (and other immune molecules, *e.g.* hemocyanin, peroxinectin and AMPs) they act as immune protein synthesis cells, suggesting a possible synergistic pathway of rapid cellular-humoral response [4]. Other tissues have shown relevant participation in the expression of some immunity lectins, such as gills, which specifically expresses LvCTL-br [59].

Overall, the expression of immunity lectins has been reported in thirteen different tissues of *L. vannamei.* The majority of these lectins are expressed in multiple tissues, such as LvLectin-2, LvCTL4 and LvPLP [11,44,60]. The multisite expression of these molecules strongly suggests their participation in fast and nonspecific response pathways, facilitating their action against infection in different shrimp tissues.

5.2. Expression patterns after pathogen challenge

During an infectious process, the host immune response strategically modulates the expression of several genes, optimizing the production of defense molecules [70,71]. The lectins are known to act on signaling and molecular traffic pathways, and these functions are targets of immunological regulation [15,72]. Consequently, many lectins of *L. vannamei* have their expression level upregulated in the presence of pathogens.

Until this moment, most experimental challenges was been conducted either with *Vibrio* sp. or WSSV. The studies show that lectins are upregulated after challenges with either of these pathogens, presenting specific expression changes according to time and tissue [14,15] (Table 3). LvLectin2, LvCTLU and LvLec1 are increased after WSSV, at 6, 24 and 12 h post infection (hpi), respectively [60,64,67]; LvPLP, LvGal and LvLTLC1 are increased, all of them at 6 hpi, after injection with *Vibrio* sp [11,33,68].

Interestingly, some lectins were also upregulated in the presence of component molecules from cell membranes of bacteria and fungi, such as LPS and Poly (I:C). LvCTL3 and LvLdlrCTL have their expression level upregulated at 4 and 72 h post exposure, respectively. Confirming that lectins act as PRRs and activate the immune response by recognizing PAMPs [43,66]. While most lectins recognize specific PAMPs, some lectins can have a broader spectrum. Studies with LvCTL5 showed higher expression level in shrimps infected with WSSV, *Vibrio parahaemolyticus* (G^-), *Staphylococcus aureus* (G^+) and *Aspergillus niger*, after 4 hpi; suggesting that the lectins can be involved in more than one signaling pathway of the immune system [46].

The antiviral action of *L. vannamei* lectins has been demonstrated mainly in shrimp experimentally infected with WSSV. However, LvCTL1 has shown increased expression levels in shrimp naturally infected, during WSS outbreaks [73]. Another lectin, LvCTL-br, was upregulated in shrimps infected with the IHHNV [59]. These observations indicate that the immunological lectins of *L. vannamei* play an important role in the antiviral defense during natural and experimental infections.

6. Immune functions of the lectins in *L. vannamei*

6.1. Induction of prophenoloxidase activating system

Since shrimps lack an adaptive immune system, the nonspecific response enzyme cascades play an important role in melanization and coagulation, providing a rapid response system against injuries [74,75]. Among these enzymatic cascades, the prophenoloxidase (proPO) activation system is responsible for the formation of nodules and encapsulation, as well as for catalysing the production of antimicrobial molecules and melanine, resulting in pathogen elimination [75–78].

The proPO activation cascade is usually activated after an injury or infection process, triggering a series of biochemical reactions [76,78]. These reactions involve several intermediate molecules, such as proteins from the serine proteases group [5,75,78]. Serine proteases work as proPO activating enzymes (PPAEs) and at the same time stimulate hemocytes degranulation, favoring the exocytosis of other antimicrobial molecules, such as peroxynectin, transglutaminase, AMPs and others, which help defense against pathogens [4,77]. The melanization resulting from activation of proPO is the final result of a complex and highly immunoeffector enzymatic process [75–78].

Often, the proPO system is activated by the presence of PAMPs that interact with PRRs, such as lectins, serving as a trigger for the cascading biochemical reactions [3]. For L. *vannamei*, two lectins seem significantly involved in the activation of the proPO system, LvCTLD and LvLec. LvCTLD was able to activate the proPO system *in vivo* and *in vitro*, increasing the encapsulation and melanization by hemocytes [47]. LvLec seems able to induce PO release through the cGMP-PKA pathway, and to influence other immunological functions as well [62,79].

Although LvCTLD and LvLec perform similar functions, these lectins are structurally different and are not phylogenetically close (see Fig. 3). Apparently, LvCTLD and LvLec act by different routes to activate the same immunological mechanism (proPO activation). LvCTLD displays an LDLR region in its N-terminal and a CRD in its C-terminal [47]. This lectin probably acts as an opsonin, mediating the interaction between hemocyte receptors and PAMPs, activating triggers for degranulation and proPO release. Molecules with an LDLR region are known to be involved in the activation of cell signaling pathways [80].

LvLec, in turn, displays the EPN (Glu^{119}-Pro^{120}-Asn^{121}) and WYD (Trp^{139}-Tyr^{140}-Asp^{141}) motifs [63], indicating that its CRD is capable of establishing more than one intermolecular interaction, thus suggesting its action as opsonin, similarly to LvCTLD. LvCTLD displays the QAP (Gln^{273}-Ala^{274}-Pro^{275}) motif [47]. EPN and QAP are characteristic mannose and galactose binding sites, respectively [29]. As Gal and Man are carbohydrates components of the O-chain of bacterial LPS, the specific binding of lectins reinforces their activity as opsonins [81]. The lectin-carbohydrate interaction depends on composition and conformation, being unique to each lectin.

6.2. Regulation of gene expression

Considering their multiple roles, some lectins are likely involved on more than one immune function, and could participate on regulation of expression and translation pathways of other immune system molecules. After *LvCTL5*-silencing, many AMPs have shown upregulation expression, such as penaeidins (*LvPEN2* and *LvPEN3*) and ALFs (*LvALF1*, *LvALF2*, *LvALF3*, and *LvALF4*); however, other lectins (*LvCLT3*, *LvLT* and *LvLec*) were down-regulated, suggesting divergent activation pathways of AMPs and lectins during recognition of pathogens [46].

Still on the topic of lectin interactions, silencing *LvGal* promoted higher expression of *Lvp53*, *LvproPO*, *LvPEN3* and *LvCrustin*, indicating that these molecules could have compensatory immunological action, due to the lack of LvGal [69]. Lectins and AMPs act in a similar way and therefore the organism may express more *PEN* and *Crustin* to supply the lack of *LvGal*, which consequently increases the level of hemocytic degranulation and ends up activating the proPO cascade more intensely

[4,69,76]. The results also suggest that the p53 cellular communication pathway is more recruited after galectin silencing, reinforcing that LvGal participates in extracellular signaling structures, probably as a cellular receptor.

In another study, the knockdown of *LvLdlrCTL* increased the expression of *LvCLT4*, *PEN4*, *Domeless* and *c-JUN*, while reduced the expression level of *ALF3, PEN3*, *P38*, *MAPK14*, *Vago4* and *c-JNK*, suggesting the involvement of this lectin in specific signaling pathways during an infectious process [66]. LvLdlrCTL shown an LDLR domain, which allows it to interact in a particular way with PAMPs and with receptors on the surface of hemocytes [66,80]. LvLdlrCTL probably induces cell signaling via the c-JNK pathway, which is depleted after its silencing and induces other types of immune responses via the c-JUN pathway, such as the synthesis of other lectins (LvCTL4) and peneidins (PEN4). The *MAPK14*, *p38* and *Vago4* are closely related to specific cell signaling pathways and probably followed the down-expression of *c-JNK*.

On the other hand, silencing *LvPLP*, unlike other lectins, promoted the down-regulated transcription of AMPs (*ALF1*, *ALF2* and *ALF3*) and phagocytosis-related genes (*peroxinectin*, *mas-like protein* and *dynamin*) [11]. LvPLP likely influences the intensity of the innate immune response, mainly by regulating gene cascades related to AMPs, acting on different pathways than those previously reported for *L. vannamei* lectins.

6.3. Antibacterial and antifungal response

Agglutination is one of the most primitive and efficient antimicrobial strategies developed during the evolution of innate immune system [1]. The lectin-mediated agglutination consists of the recognition and binding of carbohydrates present on microorganism cell surfaces, such as LPS and PGN [10,19] (Table 4).

Some lectins have a broad recognition spectrum, such as LvLdlrCTL and LvCTL5, which were able to agglutinate G^- bacteria (*Vibrio parahaemolyticus*), G^+ bacteria (*Staphylococcus aureus*) and fungi (*Aspergillus niger*) [46,66]. LvLTLC1 was able to agglutinate seven G^- bacteria (*Vibrio* spp. and *Pseudomonas* spp.) and one G^+ bacteria (*S. aureus*) [33,82–84]. These three proteins exemplify the comprehensive immunological role that a single lectin can play in the recognition and elimination of pathogens. Other lectins seem to have a smaller agglutination spectrum, such as LvPLP and LvGal, which, although binding to G^+ and G^- bacteria, are capable of agglutinating only G^- bacteria (*Vibrio* spp.) [11,68]. Finally, LvCTLU was able to agglutinate G^- bacteria (*V. parahaemolyticus*) and G^+ bacteria (*S. agalactiae*), and LvLec agglutinated *Escherichia coli* [63,67].

Flow cytometry and biochemical analyzes revealed that some *L. vannamei* lectins, such as LvCTLU, LvGal and LvLec [67,68,79], are able to induce phagocytic activity as well. Phagocytosis is described as an endocytic process of recognition and elimination of foreign particles, being highly conserved among multicellular eukaryotes [83,85]. Among invertebrates, phagocytosis is one of the main immunological pathways, involving signaling mechanisms between cells, humoral molecules (*e.g.* lectins), PAMPs and enzymatic cascades [84]. When a lectin detects a PAMP, several physiological processes are triggered to induce phagocytosis [85], such as increased production and activation of hemocytes [47], agglutination of microorganisms and increased recognition surface (opsonization) [46,47,86]. LvLec is also capable of promoting bacteriolysis of *V. harveyi* through the cGMP-PKA signaling pathway, as well as of inducing the release of PO mentioned above, exemplifying the role that some lectins may play in activating a wide diversity of nonspecific immune responses [79].

Although many of the *L. vannamei* lectins present the same binding motifs, affinity for the same molecular components and perform similar immunological functions, there is not necessarily a structural correlation

Table 4
Biochemical characterization of L. vannamei lectins.

Lectin families	Name	Method of lectin obtention	AA	MW	Substrates/Ligands	Agglutination	Ca^{2+}	Reference
C-type	LvL	Affinity chromatography (fetuin-agarose) of serum	–	172.0	NeuAc, GlcNac, GalNac, BSM, fetuin, albumin, and LPS	G^-	Yes	[61]
	LvLT	RACE followed by bioinformatics analysis	345	37.2	Galactose and mannose	–	Yes	[48]
	LvLec	Recombinant expression by *E. coli* BL21 (DE3)	157	18.0	Mannose	G^-	Yes	[62,63]
	LvCTL1	Recombinant expression by *E. coli* M15 (pREP4)	156	17.9	Mannose and glucose	–	Yes	[49]
	LvCTL-br	cDNA sequencing followed by bioinformatics analysis	347	38.5	Galactose and mannose	–	–	[59]
	LvLec1	Recombinant expression by *E. coli* BL21 (DE3)	169	18.8	Mannose, glucose, galactose and N-acetyl-D-mannose	G^+, G^-	Yes	[64]
	LvCTLD	Recombinant expression by *E. coli* (Rosetta)	311	34.6	Galactose	–	No	[47]
	LvLectin-1	RACE followed by bioinformatics analysis	156	17.4	–	–	–	[60]
	LvLectin-2	RACE followed by bioinformatics analysis	162	18.1	Galactose	–	–	[60]
	LvCTL3	Recombinant expression by *E. coli* (Rosetta) (DE3)	163	18.7	–	G^+, G^-	Yes	[43]
	LvAV	Recombinant expression by *E. coli* BL21 (DE3)	176	18.0	–	–	–	[65]
	LvCTL4	RACE followed by bioinformatics analysis	156	18.4	–	–	–	[44]
	LvLdlrCTL	Recombinant expression by *E. coli* BL21 (DE3)	303	34.6	–	G^+, G^- and fungi	Yes	[66]
	LvCTL5	Recombinant expression by *E. coli* BL21 (DE3)	171	17.1	–	G^+, G^- and fungi	Yes	[46]
	LvCTLU	Access to L. *vannamei* Genome Sequencing Project (Genbank assembly access: GCA_002993835.1) followed by bioinformatics analysis	170	18.8	–	G^+, G^-	Yes	[67]
	LvPLP	Recombinant expression by *E. coli* BL21 (DE3)	179	20.7	LPS and PGN	G^-	Yes	[11]
	LvCTL 4.2	Recombinant expression by *Sf9* cells	232	25,7	–	G^+, G^-	–	[45]
L-type	LvLTLC1	Recombinant expression by *E. coli* BL21 (DE3)	329	34.7	–	G^+, G^-	No	[33]
Galectin	LvGal	Recombinant expression by *Pichia pastoris* GS115 and *E. coli* BL21 (DE3)	338	34.0	LPS, LTA and PGN	G^-	No	[68,69]

Abbreviations: RACE, rapid amplification cDNA ends; NeuAc, N-acetylneuraminic acid; GlcNac, N-acetylglucosamine; GalNac, N-acetylgalactosamine; BSM, bovine submaxillary mucin; LPS, lipopolysaccharide; PGN, peptidoglycan; LTA, lipoteichoic acid. The molecular weight was confirmed by ExPASy (https://web.expasy.org/compute_pi/) [87].

between them, which is an evolutionary strategy to protect the host in the face of constant adaptation of pathogens to the host's immune system [12]. The structural diversity and the greater number of binding motifs are crucial for the performance of lectins as PRRs, increasing the ability to recognize glycoconjugates on the surface of pathogens [12,72]. The evolution of coding lectin genes probably comes from multiple ancestor genes, thus explaining the high number of lectins and the low homology between lectins for the same species [12].

6.4. Antiviral response

Some lectins have presented antiviral activity, and the majority of these lectins are reportedly anti-WSSV, acting in a number of different ways. LvCTL1, for example, was able to interact with structural proteins of WSSV, such as VP28, VP26 and VP24, binding the envelope proteins and rendering the viral particles unviable [49]. On the other hand, previous treatment with some lectins seem to have a protective effect in shrimps, increasing the survival rate; treatment with lectin-specific dsRNA reduced the survival rate, as in the case of LvCTL3, LvLec1, LvLdlrCTL, LvCTLU and LvCTL5 [43,46,64,66,67]. Those proteins work against viruses as PRRs, involved in non-self-recognition and regulation of other immune mechanisms.

The injection of rLvAV in shrimps challenged with WSSV has increased the virus load in the first hours, while reducing it drastically after 48 h, suggesting that LvAV hinders virus replication in an indirect way, likely through activation of other response pathways [65]. LvCTLD has shown anti-YHV activity [47]. Serology ELISA tests revealed that LvCTLD, in presence of hemolymph, was able to bind to YHV particles, while additional results suggest that LvCTLD is also involved in recruitment of hemocytes, encapsulation, melanization and phagocytosis [47].

7. Conclusion and prospects

The present review focused on the lectins that play a role on the immune system of *L. vannamei*, emphasizing the importance of these molecules for the protection of this highly farmed shrimp species against pathogens of different etiologies. It was also noticed a concentration of studies around experimental infection with WSSV and *Vibrio* sp., and fewer studies concerning other pathogens relevant for shrimp farming. This article aimed at highlighting the immunological importance of lectins for *L. vannamei*, and it will serve as a basis for research that seeks further understanding on the mechanisms involved in shrimp immune system and the action of specific molecules.

CrediT autorship contribution statement

Jhonatas Teixeira Viana: Term, Conceptualization, Writing – original draft, Writing – review & editing. Rafael dos Santos Rocha: Supervision, Writing – review & editing. Rodrigo Maggioni: Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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