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Sreeja Lakshmi *Editors*

Aquatic Lectins

Immune Defense, Biological Recognition and Molecular Advancements

Springer

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I dedicate this book to my parents who taught me to face life with enthusiasm and perseverance and whose encouraging words instigated me to pursue my dreams and succeed in life.

Preetham Elumalai

Foreword

It is a great pleasure to write a foreword for the book *Aquatic Lectins: Immune Defense, Biological Recognition and Molecular Advancements*" edited by Dr. Preetham Elumalai, Dr. Baskaralingam Vaseeharan, and Dr. Sreeja Lakshmi. I am sure that this book will prove to be good reading material for upcoming academicians, researchers, and students.

The curiosity and questions put into a chain of series end up in novel findings that open up new fields of science. The traditional aspect is extended nowadays by contemporary knowledge on methodologies renovating the applicability of advanced and novel ideas of young scientific minds. The success of a study is when it reaches towards the betterment of society. Lectins, indeed a new category, are found to be a highly debated scientific component pertaining to their ability to defend the invading pathogens and conferring immunity.

Preserving healthy environments and practices to improve disease management by enhancing the innate immune response is a highly debated current topic. Lectins hold a pivotal role in disease resistance, having been identified as proteins that possess a specific carbohydrate-binding site. Apart from the widespread natural existence, lectins can also be synthesized by the recombinant technology.

I found the vision of the book very innovative in applied science pertaining to modern knowledge that guides young researchers to take up research activities in the related area in order to extend the basic knowledge on advanced immunological parameters contributed by lectins for researchers/academicians working in the current area.

I am sure this book will captivate a wide readership, and I appreciate the sincere efforts by the authors and editors for bringing this compendium.

Shri. Parshottam Rupala
The Hon'ble Minister for Fisheries,
Animal Husbandry & Dairying
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Foreword

I consider it a great privilege to have an opportunity to pen this foreword for the book entitled *Aquatic Lectins: Immune Defense, Biological Recognition and Molecular Advancements*. I sincerely believe this important volume must be the output of the long years of research experience in the subject and concerted efforts of Dr. E.-P. Preetham and co-editors Dr. B. Vaseeharan and Dr. Sreeja Lakshmi. The book provides an overview of lectins with special reference to their therapeutic applications and emphasizes their sweeping development in immune defense properties. Each chapter of this book is intended to provide specific aspects of lectins, and I am sure readers will be able to explore the basics of the elaborative biological functions of aquatic lectins. I truly complement the authors and editors for their efforts to gather and integrate all related information for the successful outcome of this book.

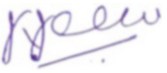
Ever since the term Lectin was coined by Prof. Boyd during the 1960s, the research on lectin, particularly its function in the immune system, witnessed phenomenal growth. Lectin is widely distributed in bacteria, fungi, viruses, plants, and animals. Even the skin of several animal species, including fish, is identified to be a rich source of novel and new unreported lectins. In addition to this, skin mucus, stomach, liver, intestine, gills, eggs, and plasma of fish are reported to have lectins.

Different types of lectins such as ficolins, galectins, calnexin, pentraxin, F-type lectins, intelectins, and mannose-binding protein (MBP) are known to play

important roles in innate immunity and disease resistance. Other main functions of fish lectins are agglutination of bacteria, fungi, parasites, and viruses, immobilization with complement-mediated neutralization and death of pathogens.

Lectins not only have roles in cellular recognition but also interact with carbohydrates. The complement system plays a vital role in protecting against invading microorganisms and acts through three activation pathways: the classic, alternative, and lectin pathways. In the lectin pathway, upon binding of the Mannan-binding lectin serine protease (MASPs) complexes to carbohydrates on the surface of pathogens, MASPs are activated and cleave the complement components C4 and C2. This results in the elimination of pathogens after a chain reaction of proteolysis of complement components and protein assembly. However, this defense mechanism is poorly understood in fish. Therefore, identification of immune-related genes and studying their expression patterns during these pathways are imperative. The book comprehensively provides up-to-date information on lectin-immune system pathways and immune gene expression analysis.

I truly appreciate the authors for their scholarly contributions to cover a wide range of aspects of lectins including their classifications, functions, and characterizations. I am sure the book will excite, educate, and encourage the next generation of researchers in exploring the ever-expanding field of lectin research.



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Foreword

The discovery and characterization of the first lectin (named "agglutinin" at the time) from the edible snail *Helix pomatia* by Camus in 1899 promoted a series of follow-up studies, initially in aquatic invertebrates and later in vertebrates, that eventually led to the realization that carbohydrate-binding proteins are ubiquitous in animal taxa. Although the initial studies were focused on the carbohydrate specificity of the newly discovered proteins, the lack of true immunoglobulin antibodies in invertebrate species suggested that they may represent their functional analogues as recognition proteins involved in defense against microbial pathogens. Since then, this book *Aquatic Lectins: Immune Defense, Biological Recognition and Molecular Advancements* edited by Drs. P. Elumalai, V. Baskaralingam, and S. Lakshmi is the first collection of review articles focused on lectins from aquatic organisms encompassing taxa from algae to invertebrates and fish, and their roles in innate immunity.

Lectins are carbohydrate-binding proteins that among multiple biological functions play key roles in innate immunity. For example, multivalent lectins can recognize a wide range of microbial pathogens, immobilize them by cross-linking, and promote their uptake and killing by phagocytic cells. Lectins are characterized by a carbohydrate recognition domain (CRD), and based on their structural fold and unique sequence motifs in their CRDs, these proteins are classified into several families, such as C-, P-, F-, R-, and I-types, galectins, ficolins, and pentraxins, all

known to play important roles in a variety of functions. The binding properties towards microbial surface structures have led to the inclusion of some lectins as pattern-recognition receptors (PRRs), a heterogeneous group of molecules that recognize microbial-associated molecular patterns (MAMPs) shared by broad classes of microorganisms. As cell surface components are essential for the microbe's survival, MAMPs are highly conserved among different microbial pathogens, and their structural features are widely recognized by innate immune receptors in their potential hosts.

Aquatic animals rely on a variety of recognition and effector factors for defense against potential infectious agents. Invertebrates lack the typical adaptive immunity of vertebrates characterized by immunoglobulins, B and T cells, and mostly rely on diverse repertoires of lectins, antimicrobial peptides, and other innate immune factors for defense against viral, bacterial, parasitic, and fungal infection. Further, although both cartilaginous and bony fish display most components of the adaptive immunity of mammals, they also depend on lectins for the rapid and effective responses targeting invading microorganisms. In both invertebrates and vertebrates, the recognition properties of lectins are amply complemented by their effector and regulatory functions that enable not only the rapid destruction of the potential pathogen, for example by the complement pathway, but also the activation and regulation of adaptive immune mechanisms.

The chapters of this book systematically and comprehensively address the recognition, effector, and regulatory functions of lectins from aquatic animals and algae, as well as their impact on biomedical sciences, including applications in diagnosis and novel therapeutic approaches. The chapters have been selected based on examples of different lectin families from a variety of animal species, in an attempt to provide an integrated view of the biological functions of these proteins that have been characterized by the implementation of interdisciplinary experimental approaches to relevant examples from different aquatic taxa.

It is clear that the future discovery of novel lectins from aquatic organisms will continue to increase the current repertory of defense molecules that are active in these organisms, and contribute to our knowledge about their structural, functional, and evolutionary relationships, as well as their potential translational value for biomedicine and aquaculture. In this regard, the editors and authors of the first edition of this book have engaged in the commendable task of organizing and integrating a substantial body of information in this field, and the resulting volume will be a very useful resource for a wide readership.

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Part I
Aquatic Lectins: History and Background

Chapter 1
Aquatic Lectins: An Overview (A Paradigm)

Abigith Abraham, C. M. Rafeeq, Resiya Karim, and Abdul Salam Rubeena

Abstract Lectins are glycoproteins that are capable of binding reversibly and specifically to sugar moieties, especially the carbohydrate content of glycoproteins and glycoconjugates. Lectin could be a membrane or soluble PRR that has a pivotal role in recognition and eradication of invading microorganisms. Lectins composed of many proteins that may particularly recognize and bind sugars such as lactose, mannose, galactose, *N*-acetyl galactosamine, and *N*-acetylglucosamine, resulting in non-covalent interactions. Lectin–carbohydrate interaction is a very important part of immunity which is not only accustomed to detect pathogens, but also employed in several alternative biological processes such as cell adhesion, agglutination, opsonization, complement activation, and phagocytosis. The lectins are classified in different ways. Based on their affinity towards the monosaccharides, lectins have been grouped into five such as Galactose Binding Lectins, Fucose binding lectins, Mannose-Binding Lectins, Sialic acid-binding lectins, and N-acetyl glucosamine binding lectins. On the basis of sources, they can be classified as plant derived lectins, invertebrate lectins and vertebrate lectins.

Keywords Aquatic lectins · Hemagglutination · Carbohydrate recognition domain · Mannose-binding lectin

Abbreviations

CRD	Carbohydrate recognition domain
CTLs	C-type lectins
MBL	Mannose-binding lectin
PRR	Pattern recognition receptor
SBL	Sialic acid-binding lectin

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1.1 Introduction

Lectins are immunoproteins that can bind to carbohydrate moieties, especially to the sugar content of glycoproteins and glycoconjugates (Dong et al. 2004). They constitute a bunch of carbohydrate binding protein molecules having an ability to interact with specific sugar moieties and conglomerate cells by interacting to glycoconjugates on the cell membranes (Saraiva et al. 2011). Lectin could be a membrane or soluble PRR that plays a pivotal role in recognition and eradication of microorganism entering into the living organism (Yu and Kanost 2004). Ricin, purified from the seeds of *Ricinus communis* and acacia from the *Abrus precatorius* extract were the first lectins isolated from plants. They are heterodimeric proteins in which two polypeptide chains are linked together by means of disulfide bonds and are ribosome inactivating proteins (RIP). Both of them have an ability to agglutinate blood cells (Olsnes 2004; Bayer et al. 2012).

The term "Lectin" (derived from the Latin "lectus," meaning "selected") was first introduced by Boyd and Shapleigh to indicate the category of proteins that have selective characteristics when interacting with carbohydrates. The term has been summarized by Sharon and Lis 1972 to incorporate all proteins from different sources, non-immune sources, and capable of binding carbohydrates, irrespective of whether or not they are peculiar for people red blood cells (Santos et al. 2013). As a non-immune source (glyco) protein, they are reversibly interacts with carbohydrates, using their binding sites, precipitates animal cells, plant cells or glycoconjugates.

Lectins confer with many proteins that may particularly recognize and bind with sugars such as galactose, mannose, lactose, and *N*-acetyl galactosamine, resulting in non-covalent interactions (Soanes et al. 2004). Lectin–carbohydrate interaction is a very important part of immunity which is accustomed to detect pathogens. Moreover, they can also be employed in several alternative biological activities such as opsonization, phagocytosis, cell adhesion, agglutination, and complement activation (Vasta et al. 2011; Osorio et al. 2011). Lectins are found in wide range of life forms belonging to prokaryotes- like bacteria, fungi, mycoplasma and eukaryotes- like animals, plants and even in viruses (Lakhtin et al. 2011). Lectins from the animal sources typically have a minimum of one carbohydrate recognition domain (CRD) that can specifically interact to varied carbohydrate units found on the cell surfaces of pathogenic microorganisms (Ng et al. 2015).

Lectins are categorized mainly into five specific groups, in accordance with their affinity towards the monosaccharide: N-acetylneuraminic acid, galactose/N-acetylgalactosamine, mannose, fucose, and N-acetylglucosamine. Based on CRD structure, pattern, binding properties, and calcium dependence, animal lectins are widely categorized into 11 major families, such as C-type, F-box lectins, F-type, M-type, L-type, I-type, P-type, R-type, calnexin, chitinase such as lectins, intelectins, ficolins, and galectins. These are being classified into several families based on their chemical properties like structure of carbohydrate binding domain, sugar specificity, and requirement of divalent cations (Medzhitov and Janeway

2002). Mainly there are three types of lectins including hololectins, chimerolectins, and merolectins (Peumans and Van Damme 1995) and these lectins are characterized by the presence of several carbohydrate binding sites (Jiang et al. 2010). Studies indicate that almost all lectin genes demonstrate a varying degree of organic phenomenon under abiotic stresses, including heat, cold, salinity, and drought (Hirano et al. 2000, SpadoroTank). All genes involved in the synthesis of lectins in rice, soybean, and Arabidopsis was characterized and identified by Jiang et al. (2015). Plant lectins, like the mannose-binding lectin (MBL), are found to impede the viral attachment at the beginning of their replication (Keyaerts et al. 2007).

Microorganisms such as bacteria, fungi, protozoa, and viruses express lectins thereby providing them with several benefits like the power to bind, infect, and inhibit other microorganisms. Lectins in microorganisms appear to play several important roles, like host–cell interactions, recognition in immune processes, phagocytosis, and cell adhesion. The lectin yield from fresh mushrooms are low, owing to the extremely high water content of fresh mushrooms. For example, extraction of lectins from the fruiting bodies of *Pleurocybella porrigens*, yielded only 2.6 mg/100 g (Suzuki et al. 2009), whereas edible fruit bodies from *Pholiota adipose*, *Russula lepida,* and *Inocybe umbrinella* yielded 70, 39, and 15 mg of lectins/100 g of fruiting bodies, respectively (Zhang et al. 2009, 2010; Zhao et al. 2009).

Lectins isolated from the phylum porifera are classified into several groups on the basis of their binding activities. It includes F-type, C-type, and tachylectin-like lectins, galectins. Extensive bioactivities are reported for sponge lectins including neuromodulatory, antimicrobial, mitogenic, and anti-tumor activities. Varieties of those activities can be correlated to physiological roles within the sponge, like spiculogenesis, host defense, cell adhesion, and differentiation.

Fish lectins have active role in the identification of pathogens and stimulating the reaction against pathogens by immune cells like phagocytes, facilitating the cell lysis by complement activation and enhancement of the activity of natural CD8 T cells (Hoffmann et al. 1999). Several lectins have been identified from various fishes such as eel (*Anguilla japonica*) (Tasumi et al. 2002), carp (*Cyprinus carpio*) (Fujiki et al., 2001), and rainbow trout (*Oncorhynchus mykiss*) (Zhang et al. 2000) with different specificities towards carbohydrates such as galactose (Vitved et al. 2000), rhamnose (Okamoto et al. 2005), mannose (Ottinger et al. 1999; Konstantina and Ioannis 2006), and fucose (Honda et al. 2000). Latterly, a sequence of lily-type lectins were reported from several fishes including orange-spotted grouper (*Epinephelus coioides*), large yellow croaker (*Larimichthys crocea*), Bartail flathead (*Platycephalus indicus*), and Spotnape ponyfish (*Leiognathus nuchalis*).

1.2 Types of Aquatic Lectins

Lectins are a diverse family of proteins or glycoproteins ubiquitously present in nature and are reported from different groups of living organisms including bacteria, fungi, animals, plants, and even in mycoplasmas and viruses (Barre et al. 2019).

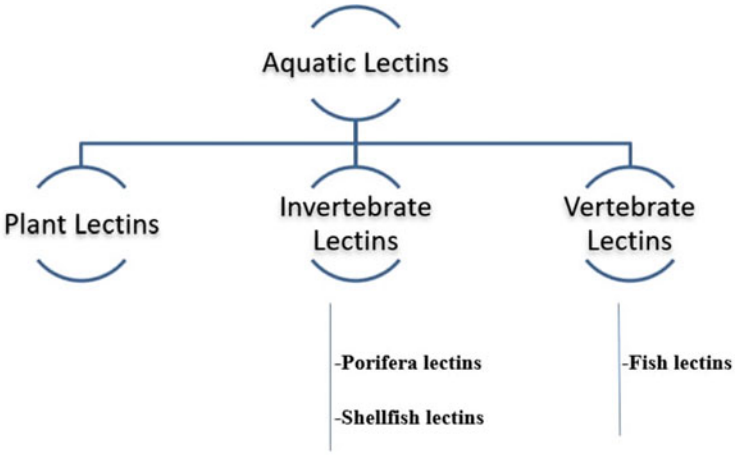

Fig. 1.1 Overview of aquatic lectins

Lectins have a characteristic affinity towards carbohydrates(Marques et al. 2018). The lectins can be classified in different ways. Functionally they are classified into five groups such as galactose-binding lectins, mannose-binding lectins, fucose binding lectins, N-acetyl glucosamine binding lectins and Sialic acid-binding lectins, which is based on the lectins affinity towards a monosaccharide (Barre et al. 2019). Based on the source, they can be classified as plant derived lectins, invertebrate lectins, and vertebrate lectins (Fig. 1.1).

1.2.1 Aquatic Plant Lectins

Plant lectin research started in 1888 by the identification of a highly toxic protein from the castor bean (*Ricinus communis*) named as Ricin and later turned out to be the first lectin (Stillmark 1888). It is followed by the extensive mining of plants for the identification of lectins and many lectins were identified from terrestrial plants, but very few lectins have been identified in aquatic lectins. It includes the recently identified lectins from *Lemna minor* or duckweed and *Eichornia crassipens* or water hyacinth. The duckweed lectin is a sialic acid lectin and the water hyacinth lectin is *N*-acetyl-glucosamine/*N*-acetyl-galactosamine binding lectins (Córdoba-Aguilar et al. 2018).

1.2.2 Algal Lectins

The first report of lectins in algae was made by Boyd et al. (Boyd et al. 1997), Rogers et al. (Rogers et al. 1977) and Matsubara et al. (Matsubara et al. 1996). Algal lectins are often termed as phycolectins and they are similar to plant lectins but there is some difference in terms of their physical and chemical properties and unique carbohydrate specificity (Singh et al. 2015). Phycolectins are identified in the red, green, and brown algal groups. More than 800 lectins were identified from algae among which 61% of them are from red algae, 22% from green algae, and 17% from brown algae. Even though these many lectins have been identified, less than 40 lectins are purified and sequenced yet (Hwang et al. 2018; Singh et al. 2015). Algal lectins share some common attributes such as low molecular weight, thermostability, independence on divalent cations for hemagglutination, and high amount of acidic amino acids. Moreover they have high affinity towards glycoproteins (Hori et al. 1990; Rogers and Hori 1993). Consequently, phycolectins are monomeric proteins having low molecular mass with an isoelectric point (pI) lies in between 4 and 6 and studies on biological models revealed that, they may be less antigenic (Teixeira et al. 2012).

1.2.3 Sponge Lectins

The exploration of phylum porifera for the identification of lectins begun during the second half of 1980, and about 50 lectins have been isolated and biochemically characterized from this species so far (Sousa et al. 2021). During this period, lectins with a wide variety of biological activities like mitogenic, cytotoxic, antimicrobial, and cytotoxic activities have been isolated. Their involvement in the immune responses in order to defend sponges from their invaders and there active role in the grouping of sponge cells was also described (Prokop et al. 1968; Müller et al. 1979). Lectins belong to the class F-type lectins, galectins, C-type lectins, and unclassified types have been identified in sponges, among which galectins are the widely seen in phylum porifera(Sousa et al. 2021). Interest in the sponge was increased recently, as they represent a stable and permanent replica of a accepted marine microecosystem having an intricate multifariousness of microbes (Gardères et al. 2015).

Even though about 50 sponge lectins were isolated and most of them have partially purified, complete determination of primary structure had been completed for only eight of them. Elucidation of their structure may provide a better understanding of their biological activities (Buck et al. 1992; Carneiro et al. 2013a; Funayama et al. 2005; Gundacker et al. 2001; Pfeifer et al. 1993; Sousa et al. 2021; Ueda et al. 2013; Wiens et al. 2003, 2005). Table 1.1 lists the lectins isolated from the phylum porifera till now.

Table 1.1 Lectins isolated from sponges

SI. no.	Name	Species	Reference
1	CcL	*Chondrilla caribensis*	Sousa et al. (2021)
2	AfL	*Aplysina fulva*	Carneiro et al. (2019)
3	AvL	*Aphrocallistes vastus*	Wu et al. (2019)
4	HoL-18	*Halichondria okadai*	Hasan and Ozeki (2019)
5	AdL	*Axinella donnani*	Ratheesh and Rauf (2018)
6	Halilectin 3 (H-3)	*Haliclona caerulea*	do Nascimento-Neto et al. (2018)
7	SfL	*Stylissa flexibilis*	Hung et al. (2018)
8	AlL	*Aplysina lactuca*	Carneiro et al. (2017)
9	Clathrilectin	*Clathrina clathrus*	Gardères et al. (2016)
10	CchG 1	*Cinachyrella sp.*	Ueda et al. (2013)
11	CchG 2	*Cinachyrella sp.*	Ueda et al. (2013)
12	Halilectin 1 (H-1)	*Haliclona caerulea*	Carneiro et al. (2013b)
13	Halilectin 2 (H-2)	*Haliclona caerulea*	Carneiro et al. (2013b)
14	HL	*Haliclona sp.*	Carneiro et al. (2013b)
15	AcL II	*Axinella corrugata*	Dresch et al. (2012)
16	CaL	*Cinachyrella apion*	Medeiros et al. (2010)
17	HoL-30	*Halichondria okadai*	Kawsar et al. (2008)
18	AcL I	*Axinella corrugata*	Dresch et al. (2008)
19	GcG	*Geodia cydonium*	Stalz et al. (2006)
20	Sd galectin 1	*Suberites domuncula*	Schröder et al. (2006)
21	Sd galectin 2	*Suberites domuncula*	Schröder et al. (2006)
22	CvL	*Cliona varians*	Moura et al. (2006)
23	CaL	*Craniella australiensis*	Xiong et al. (2006)
24	Lb MBL	*Lubomirskia baicalensis*	Wiens et al. (2005)
25	Ef lectin	*Ephydatia fluviatilis*	Funayama et al. (2005)
26	Sd lectin	*Suberites domuncula*	Schröder et al. (2003)
27	ApaL I	*Aaptos papillata*	Bretting et al. (2002)
28	ApaL II	*Aaptos papillata*	Bretting et al. (2002)
29	ApaL III	*Aaptos papillata*	Bretting et al. (2002)
30	HcL	*Haliclona cratera*	Pajic et al. (2002)
31	AaL	*Aplysina archeri*	Miarons and Fresno (2000)
32	AlL	*Aplysina lacunosa*	Miarons and Fresno (2000)
33	CcL	*Crambe crambe*	Dogović et al. (1996)
34	HoL-1	*Halichondria okadai*	Kawagishi et al. (1994)
35	HoL-2	*Halichondria okadai*	Kawagishi et al. (1994)
36	ApL I	*Axinella polypoides*	Buck et al. (1992)
37	ApL II	*Axinella polypoides*	Buck et al. (1992)
38	ApL III	*Axinella polypoides*	Buck et al. (1992)
39	ApL IV	*Axinella polypoides*	Buck et al. (1992)
40	ApL V	*Axinella polypoides*	Buck et al. (1992)
41	PsL	*Pellina semitubulosa*	Engel et al. (1992)
42	CnL	*Chondrilla nucula*	Schröder et al. (1990)

(continued)

Table 1.1 (continued)

SI. no.	Name	Species	Reference
43	DaL	*Desmapsamma anchorata*	Atta et al. (1990)
44	CalL	*Cinachyrella alloclada*	Atta et al. (1989)
45	CtL	*Cinachyra tenuifolia*	Mebs et al. (1985)
46	HpL	*Halichondria panicea*	Müller et al. (1981)

1.2.4 Crustacean Lectin/Shellfish Lectins

Different types of lectins, including Chitinase like lectins, F-type, C-type, L-type, I-type, M-type, R-type, P-type, ficolins, galectins, intelectins, and calnexin are reported in aquatic arthropods (Wang and Wang 2013). Among these, the C-type lectins (CTLs) are highly conserved in crustaceans and are well-characterized. They acts as pattern recognition receptors which can recognize and agglutinate pathogens and stimulate their phagocytosis. Lectins role in the neutralization of pathogens, involved in bacterial, fungal, and viral infections have been reported. Aside from C-type, other lectins like L-type lectins and galectins, were also reported as important immune molecules to market phagocytes against viruses and bacteria in crustaceans. However, very few lectins from molluscs were isolated and classified with their defensive roles (Liu et al. 2020).

1.2.5 Fish Lectins

Different lectins belong to Galectins, F-type, C-type, and Rhamnose-binding lectins were identified from the fishes. Fish lectins are actively involving in the innate and acquired immune reactions against pestilential microorganisms and assisting in the establishment of favorable symbiotic interactions with colonizing microbes (Vasta et al. 2012). In addition to that they also have other functions such as agglutination and eradication of infectious agents (Ewart et al. 2001; Russell and Lumsden 2005). Among the various lectins, C-type lectins are majorly reported in fishes (Elumalai et al. 2019).

1.3 Functional Aspects of Aquatic Lectins

Lectins are proteins having affinity towards carbohydrates and are identified in prokaryotes, eukaryotes and viruses. These proteins play major role in cell agglutination and can precipitate polysaccharides, glycoprotein, or glycolipid mediating several biological processes that include glycoprotein traffic signal and clearance, cell–cell interaction, induction of apoptosis, mitogenic activity, and anti-tumor activity (Fig. 1.2). Lectins have active roles in the immune system of various aquatic

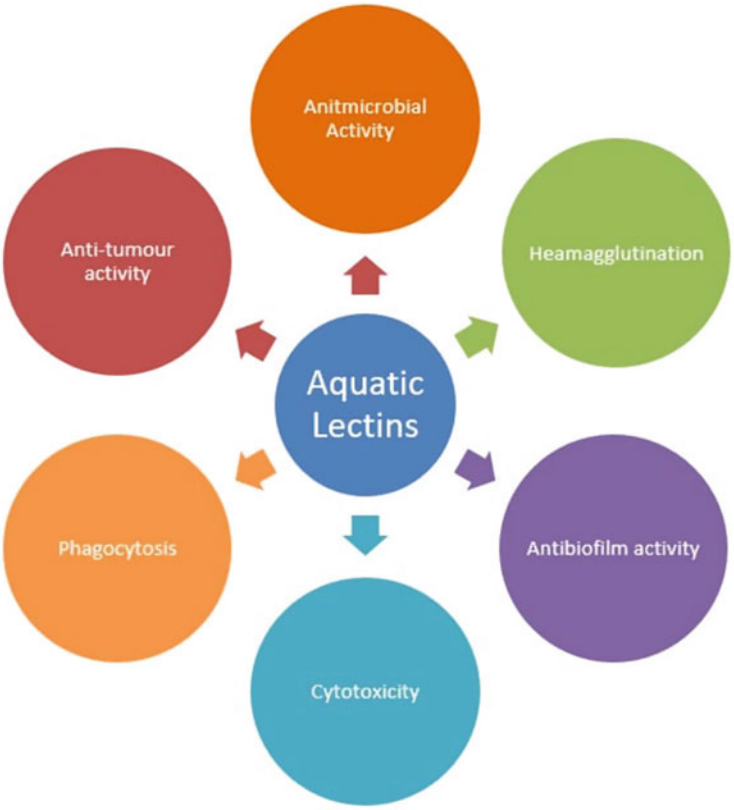

Fig. 1.2 Roles of aquatic lectins

animals, it is believed to arbitrate pathogenic recognition and plays a crucial role in innate immune reaction (Barre et al. 2019). Aquatic lectins have found to play pivotal role in processes such as embryogenesis, fertilization, and morphogenesis. Lectins can be classified into C-type lectins, F-type lectins, Galectins, I-type lectins, etc. on the basis of their structure, calcium dependency, and binding specificities. Several lectins including Calnexin, Galectins, F-type lectins, etc. are inevitable for disease resistance and in innate immunity.

1.3.1 Antimicrobial Activity

Marine sponge lectins were reported to have extensive range of biological activity. The CaL, a lectin identified in *Cinachyrella apion*, inhibited HeLa cell development and decreased cell proliferation in a dose-dependent fashion. Another lectin, CvL,

from marine sponge *Cliona varians*, reduced the development of human leukemia cells but, interestingly it had no effect on healthy blood lymphocytes.

Furthermore, lectins from marine sponges have a role in the organism's self-defense, as certain lectins may identify, agglutinate, and prevent the formation of bacterial cells and biofilms. Study on the antibiofilm activity of the lectin, AlL purified from *Aplysina lactuca*, revealed that it can agglutinate a wide range of Gram positive and Gram negative bacterial cells and thereby reduce bacterial biofilm biomass in a dose-dependent fashion (Gardères et al. 2015). A lectin isolated from *Cliona varians* (CvL), on the other hand, has shown a selective cytotoxicity against Gram positive bacteria without any effect on Gram negative bacteria.

Some lectins have cytotoxic activity against parasites and microorganisms. Lectins are agglutinating the cells by directly binding with them and in contrast, it is possible for them to act as part of a complex to exert its biological functions, as shown by the mammalian lectins. In fact lectins can bind to pathogens, by which they can either inhibit growth of the pathogen and/or kill pathogens directly. Many lectins are reported to have antibacterial activities and as the analysis used in these studies were unable to distinguish between the growth inhibition and killing, the exact mechanism of antibacterial activity is yet to be elucidated. The bacterial growth inhibitory activity of lectin is also important; whereas, further studies to characterize lectins should aim to determine whether it will kill pathogens. For example, this can be done with a fluorescent dye (like propidium iodide), which exclusively stains the cells having pores in their cell membrane.

Catfish (*Ictalurus dotatus*) have 12 galectin genes and majority of them are actively expressed in mucous tissue. Interestingly, galectin expression profiling of fish challenged with two different gram negative bacterial pathogens revealed that peculiar change has occurred in a different tissues in response to different pathogens.

1.3.2 Hemagglutination

Agglutination is the process by which cells and virus stick together or gather together. Antibodies are classic lectins and are crucial as part of both the acquired and innate immune systems. Lectins are the chief player in the innate immune mechanisms (Barre et al. 2019). Most of the lectins secreted by the mucosa have the ability to agglutinate the bacteria and exogenous red blood cells, which is termed as hemagglutination. Binding of foreign bodies (bacteria, virus, parasites, and yeasts) to lectins can prompt agglutination, which can lead to destruction (by activating complement or through endocytosis/phagocytosis uptake) or by lectin mediated direct destruction. Mechanisms like agglutination are important in preventing the absorption of pathogens through the mucosal surface. In addition to lectins, mucus also contains many immune-related molecules which can agglutinate bacteria and exogenous red blood cells. In mucus, different mucosal proteins can work in conjunction to achieve the agglutination, but in the case of recombinantly expressed or isolated lectins, they can initiate the agglutination without the aid of

other proteins, which suggests that a single lectin can cause agglutination and hemagglutination. Ability of lectin to agglutinate depends on its confirmation, which is affected by the pH, temperature and presence of ions. Hence, treating the lectin in an elevated temperature can hamper the agglutination and agglutination also depends on the presence of Ca^{2+}. Lectins possess a carbohydrate recognition domain (CRD) essentially forms a dimer or higher structure to agglutinate.

The Pufflectin, a lectin isolated from the puffer fish has no homology with other animal lectins, interestingly it has homology with plant derived mannose-binding lectins. They have been expressed from the mucosa of the oral cavity walls, esophagus, gills, and skin, etc. and reported to bind with the fluke *Heterobothrium okamotoi.* This finding suggested their active role in parasite defense. Their interactions with other bacterial species have not been tested. Hemagglutination is also very common. The mannose used in *Sebastes schlegelii* binds SsLTL lily-type lectin, the mannose of Atlantic cod (*Gadus morhua*) binds natterin-like protein, and it has been used in a calcium-dependent manner and from the flat head (*Platycephalus indicus*) mannose-binding lectin homologous to kallikrein.

1.3.3 Antibiofilm Activity

Lectins can reduce the growth and inhibit biofilm formation by several pathogenic bacterial species and thus they can have promising applications as an alternative to antibiotics to combat the increasing number of infections associated with multidrug-resistant pathogens.

Lectins identified in the marine sponge *Chondrilla caribensis* (CCL) is found to have antibiofilm activity. The analysis of antibiofilm activity of CCL showed significantly reduced total biomass on *Staphylococcus epidermidis*, *Staphylococcus aureus,* and *Escherichia coli* biofilms, but it could not reduce the viability of cells trapped in the biofilm. Biofilms constitutes sticky microbial communities covered by a macromolecular extracellular matrix produced by the microorganism itself. The resistance of the biofilm will be up to 10 ± 1000 times greater than that of the plankton cells, the protection provided by the biofilm's matrix polymers. Biofilm biomass includes not only the bacterial cells, but also biofilm substrates, which constitute for more than 90% of total biomass in most biofilms.

Chondrilla caribensis lectin activity was hampered by α-lactose, which suggests that antibiotic activity of the lectin is conferred by the carbohydrate recognition domain or CRD. Lectin exerts their antibacterial activity by the specific recognition of molecules present on the bacterial cell surface. Based on several studies, it is proven that, lectin can impede the formation of biofilms by interacting with the biofilm microbiota and altering the expression of genes involved not only in biofilm formation but also in virulence. Lectin from the haemolymph of *Metapenaeus dobsoni* (Md-*Lec*) prevents the entry and multiplication of pathogenic Gram negative organisms present in the aquatic environment. This is mediated by the inhibition of biofilm formation and agglutination of bacteria in a dose-dependent fashion.

1.3.4 Antitumour Activity

The lectins have an appreciable ability to target cancer cells which could be applied not only to kill cancers but also for the targeted delivery of anti-tumor drugs to these cells. Lectin plays central role in several processes including tissue growth, sugar storage, cell–cell interaction and communication, and other processes and pathways for cell survival and activation of immune system. Most of the endogenous lectins are involving both in physiological and pathological processes.

Eucheuma serra agglutinin (ESA), a well-characterized mannose-binding lectin isolated from the homonymous red macroalga, is capable of promoting apoptosis not only in various cell lines but also in animal cancer models. ESA consists of four tandem repeat units in its amino acid structure, which represents a binding site for the carbohydrate, mannose. All these repeated motifs specifically interact to the high mannose N glycan with minimal tetra or pentasaccharide size, such as Human (alpha13) Human (alpha1 6) Human (beta14) GlcNAc (beta14) GlcNAc.

Sialic acid-binding lectin (SBLc), alternatively termed as leczyme, purified from the egg cells of *Rana catesbeiana* is multifunctional with both lectin activity and RNAse activity. This dual nature provides them with interesting anti-tumor properties. SBLc is unique in terms of their properties and is no homologous proteins were identified yet. This is a monomeric protein having 111amino acids and contains no covalently linked carbohydrates.

1.3.5 Phagocytosis

Phagocytosis is an intricate process for the digestion and eradication of pathogens. Moreover it contributes to the fundamental homeostasis of the tissues by the elimination of apoptotic cells. The process of Phagocytosis consists of four main stages including identification of the target which leads to the triggering of signaling activity to initiate the cellular machinery for the development of phagosome, and eventually the maturation of phagolysosome. Carbohydrate–lectin interactions serve as the basis for phagocytic cells to recognize different particles and target cells**.**

C-type lectins (CTLs) are the well-studied class of lectins and hasn't undergone any mutations or changes in crustaceans. They serve as receptors for bacterial binding and agglutination or act as opsonin to induce phagocytosis of viruses and bacteria. Different CTLs involved in phagocytosis in crab and crayfish, *Litopenaeus vannamei, P. monodon*, *Fenneropenaeus chinensis, S. paramamosain, Procambarus clarkii,* and shrimp have been isolated till the date. PmLec, a lectin isolated from *P. monodon* was instrumental in recognizing *E. coli* by binding specifically to the lipopolysaccharide (LPS) present in the bacterial membrane. Furthermore, it acts as an opsonin to augment its ability phagocytize of blood cells. Several lectins with marked level of phagocytic ability against the bacteria have been identified from *L. vannamei.* Studies on the rate of phagocytosis by

V. parahaemolyticus demonstrated that silencing of lectin genes LvLdlrCTL, LvCTLU, and LvCTL5, reduced their ability by 2.5%, 4.5%, and 8.3%, respectively, compared with control groups. From these findings, it is clear that lectin produced by LvCTL5 has prominent role in phagocytosis than that of the other two lectins. Likewise, two lectins of *Paramamosain clarkii* were also able to promote phagocytic activity of erythrocytes against *Vibrio anguillarum*.

1.4 Prospective Applications of Aquatic Lectins

Lectins are ubiquitously found in the living organisms ranging from prokaryotes to eukaryotes and even in higher plants. This large group of protein is characterized by the presence of a one non-catalytic region which has the ability to reversibly bind with particular sugar moieties and their ability to agglutinate red blood cells to a specific sugar moiety is renowned. Lectins are categorized into different groups and among those C-type lectins, F-type lectins, rhamnose-binding lectins, intelectins, and galectins have been isolated from aquatic sources. .

Nowadays, appreciable attention is gained by aquatic lectins, mostly lectins from algae and cyanobacteria, because of their antiviral activity. Lectins can prevent the virus from entering host cells and spreading the virus, in contrast to the conventional antiviral therapy, in which the majority of the antiviral agents are working through the blocking of the viral life cycle after it enters into the cell. N-linked glycans present on the viral envelope has an important role in the proper folding of envelope proteins to attain its tertiary structure folding, which in turn helps the virus to enter the host cells and evasion of the host immune system. Lectins block the receptor–ligand interaction between the virion and host cells, a crucial step in the entry of the virus into the host cells, by binding with the carbohydrate residues on the viral envelope. In addition, lectins also act as surface markers for recognition of tumor cells, transmembrane signal transduction, cell adhesion, mitosis apoptosis, and cytotoxicity. Therefore, it can be used in cancer diagnosis and therapy.

Innate immunity, being the first line of defense mechanisms depends on the pattern recognition on the pathogen surfaces. Each group of microorganisms has unique molecules or patterns, which can be recognized by the patterns recognition receptors found on the macrophages, dendritic cells, and epithelial cells of the host. Studies on HSL, a lectin from sea cucumber (*Holothuria scabr)* suggests that exposure to variety of bacteria can induce its expression, which involves the interaction of glycoconjugates present on the bacterial cell wall with innate immunity receptors (Sousa et al. 2021). This result establishes the role of lectins in innate immunity. A lipopolysaccharide-binding protein named L6 isolated from limulus red blood cells, which has the characteristics of a lectin, shows agglutinating activity against the tested bacteria. Agglutination was initiated by the recognition of carbohydrate moieties present in the bacterial cell wall. It also inhibits the growth of *E. coli*, *Klebsiella pneumoniae,* and *Salmonella minnesota*.

Fungal agglutination is often studied during the characterization of lectin, as agglutination may assist its elimination through phagocytosis. Lectin's expression levels are upregulated when encountered with fungi and they normally attach to and agglutinate fungi. They act in a comparable fashion as in the eradication of invading pathogenic bacteria. Ec-CTL, A C-type lectin purified from *Epinephelus coioides* (orange-spotted grouper) also showed antifungal activity. Its expression is unregulated when stimulated with *Saccharomyces cerevisiae* and the expressed lectin bind with the fungal cells, which leads to its aggregation. The aggregation happened in a Ca2+ dependent manner. An extra cellular, serum lectin belonging to interlectins was identified in *Lampetra japonica* (lamprey) by Xue et al. (2013). It has glycan-binding receptors that attach to epithelial glycan of invading pathogens in the host system. In vivo stimulation using bacteria induced its expression and it showed agglutinative activity against opportunistic pathogenic yeast *Candida albicans*. The results suggests that it has a crucial role in the innate immune response against yeast in lamprey.

GANL, a lectin purified from the gills of bighead carp (*Aristichthys nobilis*) was tested for anti-tumor activity (Yao et al. 2003). The anti-tumor activity against six human cancer cell lines were tested, and concluded that among these, it has strong anti-tumor activity against HeLa cell lines. The IC50 value of GANL against HeLa cell line was 11.86 μg/mL. Rabelo et al. (2012) studied the apoptosis-inducing activity of sponge agglutinin in human cancer cells. They isolated CaL from the sea sponge *Cinachyrella apion* and examined its anti-proliferative effect on three different human cancer cell lines. The result demonstrated that CaL had the highest anti-proliferative activity on HeLa cells, and it was in a dose-dependent fashion. It was also reported that the lectin induced the expression of apoptotic regulator Bax, initiating the apoptosis by the activation of caspase cascade, halting the cell cycle during the S phase and improving the permeability of the mitochondrial membrane.

Conflict of Interest The authors have no conflict of interest to declare.

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Chapter 2
Aquatic Lectins: Biological Recognition Molecules

V. Ramasubramanian and V. Brindha Priyadarisini

Abstract Lectins are water-soluble and they have a role in recognition at the cellular and molecular level and play several roles in biological recognition phenomena involving cells. They also mediate attachment and binding of bacteria, viruses, and fungi to their intended targets. They found in many foods such as beans and grains. They regulate glycoprotein synthesis in animals and also regulate blood protein levels. Lectins play important role in innate immunity. Lectins are mediate pathogen recognition in fish immune system with important roles in innate immune response. Recently, animal lectin families that have already been identified and most of them occur in fish such as galectins, Pentraxins, and L-rhamnose. The structural and functional characterization of fish lectins has been approached and these studies reinforce the role of lectins in innate immune system in these animals. This chapter deals with aquatic lectins and their structural properties, animal lectins, marine and fresh water invertebrate lectins, and fish lectins.

Keywords Animal lectins · Fish lectins · Marine and freshwater algae lectins · Innate immune system · Immune absorbent assay

2.1 Introduction

Lectins are carbohydrates-binding proteins with hemagglutination activity. Lectins are found in many animals, plants, and bacteria and play an important role in the innate immune response by recognizing specific portions of carbohydrates on the surface of potential pathogens (Ajit et al. 2009). Lectins have been shown to protect plants from pathogens and also aid cell interactions in animals (Yau et al. 2015).

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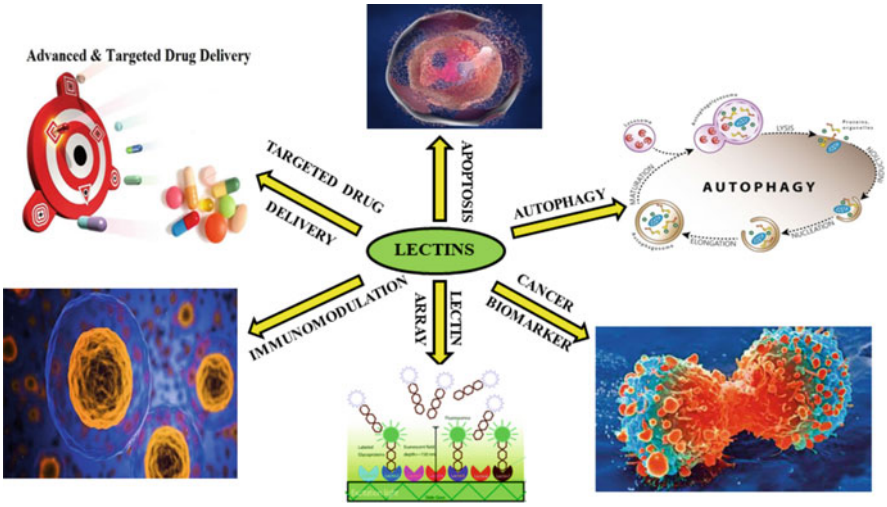
Lectins use hydrogen bonding, van der Waals forces, hydrophobic interactions, and metal coordination to bind with carbohydrate molecules. Individual lectin molecule carbohydrate binding affinities are typically much less than protein–protein interactions, with typical dissociation constants (Kd) in the milli-molar (mM) range (Goldstein et al. 1974). They have a carbohydrate recognition domain that permits them to bind to carbohydrates without being altered, indicating that they do not have any enzymatic action on carbohydrates. Because they bind with carbohydrates in a reversible way, whether simple sugars or complex carbohydrates, lectins can precipitate polysaccharides and glycoproteins as well as agglutinate cells (Nascimento et al. 2012). Cellular localization, structural and evolutionary sequence similarities, taxonomic origin, carbohydrate recognition, function, and structure are some of the criteria used to characterize lectins (Martínez-Alarcón et al. 2018). Cell–cell and host–pathogen communication, tissue development, sugar storage, and other cell survival and immune system activation mechanisms are all mediated by lectins. Endogenous lectins have been linked to physiological as well as pathological processes (Dan et al. 2015). Lectins are assumed to have an intriguing anticancer strategy because they can induce apoptosis and autophagy while inhibiting angiogenesis (Dan et al. 2015).



2.2 Identification of Lectins

Most lectins are divalent or multivalent, capable of interacting with carbohydrates in solution or when linked to cell membranes, and their binding sites interact with cells, forming a variety of reversible linkages. Lectins are easily detected using agglutination assays as a result of this property. The hemagglutination assay makes it

simple to observe lectins ability to agglutinate erythrocytes. The use of erythrocytes from various animal or human origins that were biochemically (Leite et al. 2005; Jung et al. 2007), chemically (Santos et al. 2009; Araújo et al. 2012), or insufficiently treated (Santos et al. 2009; Francis et al. 2011) confirmed that lectins may be unique to different erythrocytes. Cordyceps militaris lectin agglutinates mouse and rat erythrocytes but not ABO group cells (Jung et al. 2007); The lectin of Gracilaria ornata agglutinates animal (rabbit and chicken) but not human erythrocytes (Leite et al. 2005). PpyLL did not bind to human erythrocytes (types A*, B, or O)* and had no hemagglutinating activity (HA) with human AB erythrocytes; additionally, this lectin agglutinated rabbit but not chicken or rat cells (Costa et al. 2010). The coagulant Moringa oleifera seed lectin, cMoL (Santos et al. 2009), and the Crataevatapia bark lectin, CrataBL (Araújo et al. 2012) are two other non-specific blood group lectins. Previous hemagglutination assays have shown that inhibition of hemagglutination in the presence of carbohydrate confirms lectin detection; polysaccharide or glycoprotein precipitation assays are also used to evaluate lectin samples (Goldstein et al. 2007). Specific lectins for monosaccharides and oligosaccharides. However, oligosaccharides, glycoproteins, and/or polysaccharides are the only substances that inhibit some lectins (Walti et al. 2008; Thakur et al. 2007). Many lectins can be inhibited, including lectins that bind to rhamnose in the eggs of the *Scomberomorus niphonius* fish (Terada et al. 2007), lectins that bind to lactose in the mushroom *Agrocybecylindracea*(Liu et al. 2008), and lectins that bind to galabiose, a rare disaccharide in human tissues (Goldstein et al. 2007).

2.3 Structural Properties of Lectin

The three-dimensional structure of lectin binding sites revealed a conserved amino acid profile within lectin families, as determined by carbohydrate specificity (Peumans and van Damme 1998). The majority of the residues in these molecules coordinate the metal ions required for subunit integrity and proper residue positioning, and they share a high degree of similarity in their residues, including those involved in binding to monosaccharides (Sharon 1993). Metal ions and interactions are found in lectins, which are coordinated by water molecules and carbohydrates (Sharon and Lis 2002). Depending on the molecule and oligomerization state, these proteins can have 2 to 12 interaction sites (Balzarini 2006). Lectins differ structurally from the primary structure to the last degree of molecular organization; they may differ in amino acid sequence, number of subunits, and polypeptide nature. Interactions between subunits appear to be important in this regulatory element - binding stability (Mitra et al. 2002). The features and affinities of the associated sites are accomplished mainly through hydrogen bridges, which include van der Waals interactions and hydrophobic interactions with aromatic amino acid residues close to the hydrophobic portions of monosaccharide (Sharon 1993), which contribute to the complexes' stability and specificity.

2.4 Mechanism of Lectins

Lectins, in particular, bind to a unique sequence of monosaccharide moieties found on the cell surface in glycosylated proteins, lipids, and glycans. In humans, these proteins are expressed by a diverse range of cells, from epithelial cells to antigen-presenting cells, and depending on the type of lectin, they can be exposed on the cellular membrane or secreted into the extracellular matrix (Drickamer 1988). Typically, each lectin has multiple sugar unit binding sites. As a result, they can bind to different carbohydrates on the surface of different cells, allowing for cell–cell interaction (Santos et al. 2014). The distinct glycosylated proteins expressed in tumor cells become the target of lectins, which bind to the distinct glycan chains. During tumorigenesis, specific subsets of glycans on the cancer cell surface undergo various changes, such as enrichment or decrease in their main components. Sialylation enrichment, branched-glycan structures, and the formation of the so-called fucosylation core are the most common changes to cell surface glycosylation patterns (Pinho and Reis 2015; Munkley and Elliott 2016).

2.5 Animal Lectins

Animal lectins have a wide range of biological functions. The presence of lectins in invertebrate hemolymph and coelomic fluid is found in almost all phyla. Protozoa (Brown et al. 2007; Heron et al. 2011), insects (Ourth et al. 2005), mollusks (Banerjee et al. 2004; Takahashi et al. 2008), crustaceans (Yang et al. 2007; Sun et al. 2008; Sanchez-Salgado et al. 2014), sea cucumbers (Gowda et al. 2008a), polychaetes (Molchanova et al. 2007; Moura et al. 2006), and sea sponges (Moura et al. 2006) have all been found to contain lectins. In vertebrates, lectins from fish (Carvalho et al. 2012; Cammarata et al. 2014), snakes (Nunes et al. 2012; Aranda-Souza et al. 2014), and other animals have been isolated and characterized. Many lectins have been identified in human tissues and cells, including the lungs (Kishore et al. 2006; Sorensen et al. 2007), serum (Bouwman et al. 2006; Wallis 2007), and dendrites (Kanazawa et al. 2004; Kanazawa 2007). In vertebrates, lectins are divided into two types based on where they are found: membrane integral lectins and soluble lectins found in intra and intercellular fluids (Barondes 1984). Membrane structural components with varied carbohydrate specificity, as well as physical and chemical properties, are known as integral lectins. Lectins that are soluble can travel freely within and between cells. Antibacterial (Araújo et al. 2012; Francis et al. 2011), antifungal (Bhutia et al. 2019), and immunomodulatory properties are shared by these compounds. C-type lectins, I-type lectins, galectin (or S-type), pentraxins, and P-type lectins were classified by Gabius (1997) based on structural properties. C-type lectins contain different specificities and carbohydrate recognition domains that bind carbohydrates in the presence of Ca^{2+} ions. The carbohydrate recognition domain (CRD) of I-type lectins is similar to that of immunoglobulins. Galectins, also

known as S-type lectins, are thiol dependent lectins with a CRD that only recognize galactosides. Pentraxins are composed of many subunits that join together to form pentameric lectins P-type lectins are glycoproteins that contain mannose 6-phosphate and are specific to them, have a similar but poorly defined CRD. They also suppress angiogenesis while increasing apoptosis and autophagy (Bhutia et al. 2019; Santos et al. 2014). They may also be effective in identifying small changes in carbohydrate composition that occur during cancer transformation because of their capacity to attach to carbs (Višnjar et al. 2019). While various studies have summarized the anticancer action of terrestrial lectins (Bhutia et al. 2019; Shi et al. 2017), no thorough analysis addressing the anticancer activity of marine lectins has been published to our knowledge. Rhamnose-binding lectin (RBL) is a family of animal lectins known for their sugar-binding affinity and molecular structure, which is made up of two to three homologous, tandemly repeated CRDs (Yau et al. 2015). Rachycentron canadum (Jimbo et al. 2007) and *Oreochromis niloticus* serum contain lectins that recognize methyl-D-mannopyranoside; the latter lectin also recognizes D-mannose (Coriolano and Coelho 2012).

MBL (mannose-binding lectin) is an important component of innate immunity that can activate the lectin pathway of the complementary system. In freshwater fish, (Zhang et al. 2012; Silva et al. 2012) discovered an MBL gene (*Ictalurus punctatus*).

Endocytosis mechanisms, intracellular translocation of glycoproteins (Zhang et al. 2012), binding to glycoconjugates (Barondes 1984), apoptosis processes (Yamashita et al. 1999), defense against microorganisms, regulating cell adhesion, and migration processes, and bacterial binding to epithelial cells appear to be mediated by animal lectins (Liu et al. 2012). They also play a role in the immune systems of crustaceans, birds, and mammals (Sanchez-Salgado et al. 2014). Membrane integral lectins are thought to be involved in glycoconjugate binding to cell surface or vesicle membranes, which results in glycoconjugate localization (endocytosis) or transport to other cellular compartments (intracellular translocation) (Barondes 1984). Meanwhile, soluble lectins circulate within and between cells, interacting with other soluble substances and binding to membrane glycoconjugates. The fact that these proteins appear to be concentrated within cells before being released suggests that they all have the same function of binding to cell glycoconjugates. Mannose-specific lectins in mammalian serum are the best researched C-type lectins, which operate against pathogens by binding to oligomannosides on bacterial and fungal cell surfaces and neutralizing them by cell lysis or opsonization (Sanchez-Salgado et al. 2014). CTLR, a receptor-type lectin, functions as a cell surface signaling molecule, recognizing a variety of highly conserved pathogen molecules and stimulating an appropriate immune response (Holmskov et al. 1994). Lectins are proteins found in the cell structures of insects and protozoa (invertebrate animals); these proteins appear to be related to the method by which parasitized hosts are recognized by some protozoa. The lectin was validated as a modulator of these parasites' adherence to host tissues in immunohistochemical and immunocytochemical tests utilizing cattle tissues to discover Tritrichomonas sp. lectin binding sites (Willcocks et al. 2006).

2.6 Marine and Freshwater Algae Lectins

Chlorophyll-containing organisms are known as aquatic algae. They live in seas, rivers, lakes, and soils, and they are symbiotic partners with animals and plants. Mannose-specific lectins have been discovered in nearly every higher plant family, including monocot and dicot groups. These organisms range in complexity from simple unicellular entities to complicated pluricellular entities that reorganize to produce simple tissues. At least 44,000 algal species have been identified and named (de Oliveira Figueiroa et al. 2017), with lectins found in 250 of them (Pinho and Reis 2015). Only a few of these, however, have been thoroughly investigated to establish their anticancer potential.

The Eucheuma serra agglutinin (ESA) is the most well-studied lectin of algal origin, having been produced from the namesake red macroalga. ESA is a mannose-binding lectin that has been shown in cell lines and animal tumor models to induce apoptosis (Vermeulen 2013). Colo201 (human colon adenocarcinoma), Colon26 (murine colon-carcinoma), HeLa (human cervical adenocarcinoma), MCF-7 (human breast adenocarcinoma), OST (human osteosarcoma), and LM8 (murine osteosarcoma) are among the cancer cell lines affected by ESA (Vermeulen 2013). Apoptosis is the mechanism of cell death in each of these cell lines, as evidenced by DNA fragmentation, phosphatidylserine exposure, and caspase-3 activation. ESA had no effect on normal fibroblasts or the non-tumorigenic epithelial MCF10-2A cell line. In addition, their tumor cell selectivity leads to no damage in vivo (Hayashi et al. 2012). ESA, for example, inhibited Colon26 cell proliferation in BALB/c mice without reducing body weight or inducing death, showing promising in vivo tolerability (Hayashi et al. 2012).

ESA-tagged lipid vesicles resembling microcapsules were used in a selective drug delivery system (DDS) (Fukuda et al. 2006). The microcapsules were made from sorbitan monooleate (Span80) with or without poly (ethylene glycol) (PEG) (Vermeulen 2013; Sugahara et al. 2001). Because PEGylation should reduce reticuloendothelial absorption, PEG was added to the vesicles to increase their half-life relative to normal liposomes. To begin with, it was shown that both ESA-labeled DDSs target the same carbohydrate-sequence of free ESA, and that the drug transportation mechanism has no effect on its cytotoxic efficacy or tumor cell selectivity (Vermeulen 2013). Normal human fibroblasts and MCF10-2A displayed little interaction with ESA-immobilized lipid vesicles, which approached and interacted with Colo201, HB4C5, and OST (Vermeulen 2013). Despite the fact that ESA was ineffective against MCF-7, two isolectins isolated from Solieria filiformis (Sfl) have shown their ability to fight breast cancer. The lectin Ulva pertusa lectin 1 (UPL1) binds N-acetyl D-glucosamine and interacts with a number of intracellular pathways important in cell proliferation and survival.

2.7 Marine and Freshwater Invertebrate Lectins

Animals from the marine invertebrate population have been utilized for their biological activity since ancient Greece. Hippocrates and Galen, two forefathers of modern medicine, wrote extensively on the dietary and pharmacological applications of mussels, sponges, and cephalopods, and prescriptions incorporating marine invertebrates alongside other medicines have been unearthed. Marine invertebrates are a diverse group of organisms that have been divided into more than 30 phyla (Omokawa et al. 2010). They lack an innate immune system and do not establish a pathogen-specific adaptive immunological response.

2.8 Mollusca and Arthropoda

Mollusca are the second most populous phylum of animals in the world. Gastropoda account for over 90% of all mollusks, followed by bivalves and cephalopods (De Zoysa 2012). The shellfish family, which includes molluscs and crustaceans (phylum Arthropoda), is the most traditionally eaten medicine (Ponder and Lindberg 2008). Bivalves and crustaceans are a remarkable source of molecules, such as the unique lectin proteins that can be used as therapeutic agents, according to several studies. The abundance of hepato-pancreatic mass, the distinctive open circulatory system, filtering abilities, and shell arrangement make bivalves and crustaceans a remarkable source of molecules, such as the unique lectin proteins that can be used as therapeutic agents. CGL (Crenomytilusgrayanuslectin) is a fascinating lectin isolated from the homonymous bivalve of the Mytilidae family (Bouchet and Duarte 2006). It consists of three tandem amino acid sequences with a total of 150 residues (Fredrick and Ravichandran 2012). It has been overexpressed in a number of human tumors with intrinsic or acquired multidrug resistance (Bekri et al. 2006). CGL inhibits cell proliferation and promotes cell death in Gb3-expressing tumor cells like Raji cells (Burkitt's lymphoma) and, to a lesser extent, MCF-7 (breast carcinoma). The Mediterranean mussel, Mytilus galloprovincialis, contains a lectiMytilec that behaves similarly to CGL. It comes as no surprise that they share 50% of the amino acid sequence and bind the same glycan moiety (Behnam-Motlagh et al. 2010). Mytilec enters cells through interactions with Gb3, and as a result, it promotes cytotoxic effects. It is only antitumor in Gb3-expressing cell lines like Raji's and Ramos, another Burkitt's lymphoma cell line, and has no effect on K562 (UniProt 2019). In Ramos cells, where it induces apoptosis and activates all MAPK pathways, the mechanism of action of Mytilec has been investigated. The MAPK system is made up of three protein kinases that are activated in a certain order and play key roles in numerous transduction pathways. These pathways are involved in eukaryotic activities such as cell growth, differentiation, and death (Anam et al. 2017).

iNol is a large lectin that is physiologically synthesized by the Arthropoda slipper lobster (Ibacusnovemdentatus) to kill pathogens. iNol is made up of five subunits

that are held together by disulfide bonds and are 70-40-, or 30-kDa polypeptides. It has a polygonal structure under physiological conditions. Despite its large molecular weight, this lectin enters mammalian cells such as HeLa cells via endocytosis by attaching to N-acetylated glycan moieties on the cell surface. iNol kills HeLa, MCF-7, T47D (breast cancer), and Caco-2 (colon cancer) cells. HeLa cells were selected to investigate the apoptotic potential of iNol activity since they were the most vulnerable. Only after the lectin binds its carbohydrate-ligand does iNol enhance DNA fragmentation and caspase-3 and caspase-9 activation, suggesting that its anticancer potential is firmly tied to its lectin nature (Keshet and Seger 2010).

Tachypleus tridentatus, also known as the Chinese or Japanese horseshoe crab or tri-spine horseshoe crab, is an arthropoda that resembles a crab but is related to spiders and scorpions. Tachypleus tridentatus, despite its unflattering name, is a source of several lectins, including the rhamnose-binding Tachypleus tridentatus lectin (TTL). The characterization of TTL is not completely understood. Several studies have concluded that this lectin is a multimer, but it is unclear whether it is made up of hexamers, octamers, or tetramers (Fujii et al. 2017; Gokudan et al. 1999). The antitumor activity of TTL was tested in vivo after its genomic insertion into an oncolytic VV (oncoVV-TTL). When compared to oncoVV-only treated patients, OncoVV-TTL was able to replicate inside tumor cells and significantly reduced tumor growth (Kairies et al. 2001).

2.8.1 Porifera

Porifera means “pore bearer,” and it is the most distinguishing feature of sponges. Indeed, they are sessile invertebrates with no digestive, nervous, circulatory, or immune systems. Two lectins derived from different species of the genus Haliclona demonstrated exceptional antitumor activity. The antitumor activity of Haliclona cratera lectin (HCL) was tested on human cervical and melanoma cell lines. It exhibited cytotoxicity against both tumor models, which were nearly identical in sensitivity. Haliclona caerulea, another species, produces a unique lectin known as halilectin-3 (H3). H3 has the ability to promote cancer cell death through a variety of mechanisms. H3 activates both intrinsic and extrinsic apoptosis pathways in MCF-7 cells, promoting an early upregulation of p53 and inhibiting cell proliferation, resulting in an accumulation of cells in the G1 phase. H3 inhibited integrin 61 expression and interacted with integrin 51, the fibronectin receptor, impairing MCF-7 adhesion and promoting cell death (Li et al. 2018). H3’s involvement in cytotoxicity via apoptosis, autophagy, and anoikis makes it a very promising antitumor lectin, capable of fighting cancer on multiple fronts and thus reducing drug resistance. A lactose-binding lectin isolated from the sponge Cinachyrellaapion (Cal) demonstrated antitumor activity on human cervical (HeLa) and prostate adenocarcinoma (PC-3) cells, while non-transformed 3T3 mouse fibroblasts showed milder cytotoxicity. Hol-18 is a lectin that binds N-acetylhexosamine and was isolated from the Japanese black sponge Halicondriaokadai. Few studies have shown that it has

antitumor activity in Jurkat (acute T cell leukemia), K562 (do Nascimento-Neto et al. 2018), HeLa, MCF-7, and T47D cells.

2.8.2 *Chordata*

Chordata also contains lectins. The N-acetyl-D-glucosamine-binding Didemnum ternatanum lectin is the most compelling tunicates lectin (DTL). DTL is a homotrimer whose activity is unaffected by Ca^{2+} and Mg^{2+}. It contains relatively high levels of Gly, Ala, Asx, Glx, Leu, and Val residues, and, like the other lectin isolated from marine sponges, it is partially composed of carbohydrate (around 1.3 percent) (Matsumoto et al. 2012). Because cell–matrix interaction is one of the most important functions of lectins, the antitumor potential of DTL on HeLa cells has been studied in various microenvironment conditions. DTL behaved differently on tumor cells depending on their anchorage status. In normal adhesion conditions (cells grown in adhesion plates), DTL promotes cell growth, whereas in abnormal adhesion conditions (cells grown in adhesion plates), it inhibits cell proliferation and triggers cell differentiation in a way that promotes cell attachment (Belogortseva et al. 1998a, b). Thus, the cell microenvironment is important in DTL activity. Keeping this in mind, using tumor 3D cultures built with specific bioreactors could be an intriguing approach to better predict the anticancer potential of lectins. Standard in vitro models do not capture the complexities of tumor biology and do not take into account cell-to-cell and cell-to-matrix interactions. Perfusion-based bioreactor systems generate heterogeneous cell populations and optimal physiological cell–cell and cell–extracellular matrix interactions, mimicking the microenvironment perfectly (Odintsova et al. 2001; Fang and Eglen 2017).

2.9 Lectins from Marine and Freshwater Vertebrates

2.9.1 *Amphibians*

Amphibians are ectothermic tetrapod vertebrates with a diverse range. Recently, drug discovery has delved into amphibians' metabolites, owing to the knowledge that they produce several metabolites primarily as an essential self-defense strategy. Many anticancer peptides have been discovered in amphibian skin, oocyte cells, and eggs. A sialic acid-binding lectin (SBLc), also known as leczyme, is a multifunctional protein isolated from Rana catesbeiana oocytes that have both lectin and RNAse activity. This is the key to its intriguing antitumor properties. This lectin is distinct and not related to any other known protein. It is a single subunit with 111 amino acid residues and no covalently bound carbohydrate. The lectin-glycan binding of SBLs has not been fully elucidated. (Nitta et al. 1987; Bourgine et al. 2018) demonstrated that blocking amino groups, specifically t-amino groups, but not

tyrosine residues, inhibited Lectin hemagglutination activity, implying that those residues are responsible for the sugar-binding. The lectin component of SBLc selects cancer cells by binding to specific sialic acid carbohydrates residues; then, specific receptors allow it to enter the cells, where the RNAse activity promotes RNA cleavage. SBLc has been shown to activate the p38 MAPK pathway in leukemia, mesothelioma, and several breast cancer cells (Nitta et al. 1987). Many different types of specialized cells can phagocyte dying cells to prevent inflammation and promote their clearance. SBLc induced morphologic and phenotypic changes in MCF-7 cells, allowing them to phagocyte SBLc-triggered dying cells (Kariya et al. 2016), resulting in very efficient auto-clearance. This out of the-ordinary behavior adds to SBLc's already promising anticancer activity.

2.9.2 Fish

The food industry does not account for all of the economic value of fish. On the contrary, many studies are focusing on these animals because they produce many bioactive compounds, such as human calcitonin from salmon, which is used to treat postmenopausal osteoporosis (Yiang et al. 2012). Fishes, unlike amphibians, produce a variety of lectins that have been isolated from eggs, skin mucus, plasma, and serum (McLaughlin and Jialal 2019). The biologic function of fish lectin is unclear, despite evidence suggesting an impact on fertilization and morphogenesis, as well as a defense activity against microorganisms (Tasumi et al. 2004).

Aristichthys nobilis, also known as big head carp, is a Cyprinidae family member that includes zebrafish. GANL, a lectin that causes tumor-type-dependent cell death, is produced. GANL is a homo-multimeric glycoprotein that does not require Ca^{2+} ions to work. The carbohydrate content is approximately 13.4%, while the protein portion contains a high concentration of Asp, Glu, Leu, Val, and Lys. These amino acids form -helices, unordered structures, −turns, and -sheets on their own (Dutta et al. 2005). GANL inhibited proliferation of HeLa, SKOV3 (ovarian cancer cells), and HepG2 cells in a concentration-dependent manner but did not affect SMMC-7721 carcinoma cells or BGC803 gastric cancer cells.

The Gb3 sugar chain is specifically recognized by galoctoside binding lectins isolated from the eggs of the catfish Silurusasotus (SAL) and chum salmon eggs (CSEL). Gb3, SAL and CSEL bind to cells, are internalised, and have antitumor activity. As a result, despite several studies showing antitumor activity on Burkitt's lymphoma cells (Yao et al. 2012), SAL is inactive on Gb3-deficient K562 cells. As a result, despite several studies showing antitumor activity on Burkitt's lymphoma cells (Yao et al. 2012), SAL is inactive on Gb3-deficient K562 cells. CSEL also induces apoptosis in Gb3-positive Caco-2 cells but not in Gb3-negative DLD-1 colorectal adenocarcinoma cells. SAL induces apoptosis-like phenotypic changes in Raji cells, such as phosphatidylserine exposure and cell shrinkage (Shirai et al. 2009), but its antitumor activity is more likely linked to its ability to inhibit the cell cycle (Yao et al. 2012).

Another antitumor lectin found in Chinook salmon, also known as king salmon due to its massive size, is rhamnose-binding chinook salmon roe lectin (CSRL). Cell proliferation of MCF-7 and HepG2 cells has been reported to be inhibited by CSRL. With a double potency, HepG2 was found to be more sensitive to CSRL than MCF-7. CSRL, like all of the lectins discussed in this review, had no toxic effect on WRL68, a non-transformed liver cell line (Sugawara et al. 2005). p53 and p73 (the proline 73 polymorphic variant of p53) represent the links between EF2–1 and apoptosis (Bah et al. 2011). In Hep3b cells, two distinct lectins can activate this pathway and promote apoptosis. DIFBL-FLAG and AJL1-FLAG were created by encoding Dicentrarchus labrax fucose-binding lectin (DlFBL) and Anguilla japonica lectin 1 (AJL1) in an adenovirus with a replication defect. Several human liver and lung cancer cell lines were cytotoxic to DIFBL-FLAG and AJL1-FLAG were cytotoxic to several human liver and lung cancer cell lines (Irwin et al. 2000). DIFBL is a non-glycosylated Ca2 + −independent lectin. It is a dimeric protein composed of two protein fractions that, when separated, only one has lectin activity, whereas in physiological conditions they are stabilized by disulfide bonds (Li et al. 2016). Despite their remarkable biological activity, some lectins transported by viral vectors struggle to infect cells significantly due to a lack of specific receptors such as the coxsackie-adenovirus receptor (CAR). To facilitate this process, other oncolytic adenoviruses with a CAR-ligand-expression cassette in their genome can be used to target cell membrane receptors. As a result, after infection and replication, the expression of CAR ligand will aid the subsequent adenovirus infection, resulting in a positive feedback mechanism.

A number of isolated lectins from sea hydrobionts have been classified as C-type lectins, which have carbohydrate binding capacity that is Ca^{2+}-dependent (Wu et al. 2017). Other marine lectins, such as those in the Mytilidae family, are not included in this category (Chernikov et al. 2013). Recently, a novel lectin from the sea mollusk Crenomytilusgrayanus (hereafter denoted as CGL) was purified and characterized from the sublittoral zone of Peter the Great Bay of the Sea of Japan (Fujii et al. 2012). CGL has the ability to agglutinate all types of human erythrocytes, which can be effectively inhibited by *N*-acetyl-D-galactosamine (GalNAc), D-galactose, and D-talose (Belogortseva et al. 1998a, b). Because of its GalNAc/Gal specificity, CGL is assumed to be a member of the galactose binding lectin family. CGL's amino acid sequence, with the exception of MytiLec (Cammarata et al. 2001), exhibits minimal resemblance to galectins or other animal lectins. According to molecular modeling, CGL has a trefoil structure similar to the ricin B-like lectin. Among other proteins, the trefoil fold is found in cytokines, agglutinins, and actin-crosslinking proteins. On the other hand, these proteins have evolved to have a diverse spectrum of legend binding preferences and biological functions (Broom et al. 2012).

2.10 Properties and Applications

Lectins are unique in biotechnology because of the numerous applications and other prospective uses that are being evaluated and explored by lectinology experts on a regular basis. Lectins and their unique capabilities, which include the capacity to bind glycoconjugates, are useful tools in a wide range of scientific fields, including biochemistry, cellular and molecular biology, immunology, pharmacology, medicine, and clinical analysis. Agglutination, mitogenic stimulation, redistribution of cell surface components, changing the activity of membrane enzymes, suppression of bacterial and fungal growth, cell aggregation, toxicity, and immunomodulation are only a few of the actions of lectins on cells (Silva et al. 2015).

Specific binding lectins can detect tissues and aid in illness diagnosis based on the carbohydrate. The seed lectin of Cratyliamollis is a valuable tool for biological research (Silva et al. 2014). In clinical samples, the lectin may identify dengue glycoproteins (Avelino et al. 2014). Ohyama et al. 2004 investigated different lectins for their ability to bind to carbohydrates on serum prostate-specific antigen (PSA); they discovered that these lectins contain a prominent N-glycan core structure with a sialic acid alpha2–3 galactose linkage as an extra terminal carbohydrate. In comparison to patients with benign prostate hypertrophy, the M. amurensis agglutinin-bound fraction of free serum PSA increased in prostate cancer patients. Surface plasmon resonance research validated PSA's binding to M. amurensis agglutinin, which detects alpha2,3-linked sialic acid. As a result, the difference in free serum PSA binding to *M. amurensis* agglutinin lectin between prostate cancer and benign prostate hypertrophy could be used as a marker for prostate cancer diagnosis. Seed lectins from C. mollis were used to isolate glycoproteins from complicated protein mixtures using affinity matrices (Napoleão et al. 2013). In alloxan-induced diabetic mice, a lectin from Crataeva tapia bark reduced tissue damage and plasma hyperglycemia (Rocha et al. 2013).

2.10.1 Lectins Against Microorganisms

Different lectins have been found to exhibit activity against bacteria, protozoa, fungi, and nematodes (Wellman-Labadie et al. 2008; Moura et al. 2006; Sitohy et al. 2007) and nematodes (Ripoll et al. 2003), indicating that these proteins could be useful in future clinical therapies, (e.g.) N-acetyl-glucosamine, have a remarkable anti-HIV (human immunodeficiency virus) activity.

The study of the effects of lectins on bacteria of medicinal value is gaining popularity. As lectins identify carbohydrates or glycoconjugates in cell surfaces, they can interact with them and hinder microbial cell development. Lectins from various sources, such as a lectin from the marine species *Holothuria scabra* (Gowda et al. 2008b), a lectin from Araucaria angustifolia seeds (Santi-Gadelha et al. 2006), and C-type lectins from chicken and goose eggs (both active against *B. subtilis*,

S. aureus, and *P. aeruginosa*) (Wellman-Labadie et al. 2008), can act as antibacterial agents against Gram-positive and Gram-negative bacteria. Cladonia verticillaris lichen lectin (ClaveLL) possesses antibacterial and antifungal activity against Gram-positive (Bacillus subtilis, Staphylococcus aureus, and Enterococcus faecalis) and Gram-negative (Escherichia coli and Klebsiella pneumoniae) organisms (Wellman-Labadie et al. 2008). Plant lectins have been shown by (Ramos et al. 2014) to be capable of distinguishing possible pathogenic mycobacterial species.

2.10.2 Lectins and Cytochemistry/Histochemistry

The binding of lectins to glycoconjugates in cells, tissue sections, or free carbohydrate/glycoconjugates allows them to detect carbohydrates. Direct visualization using tagged lectins (McCafferty et al. 2008) or indirect detection utilizing immunological methods are used to detect binding. For the detection of glycoproteins and other glycoconjugates, radio-marked lectins and conjugated lectins are sensitive and specific reagents (Tazaki 1997; Szöke et al. 2007). Lectins are cytochemical, histochemical, or immunohistochemical tools for identifying glycoconjugates in various animal tissues (Thöm et al. 2007), as well as for detecting and characterization of glycosilated residues and other glycoconjugates present in human or animal cells and tissue surfaces (Franceschini et al. 2000).These proteins have been used to study kidney tissues from humans and other animals, and have made significant contributions to illness diagnosis and prognosis, including cancer. Lectins can identify prostate cancer from benign hyperplasia (Hemmoranta et al. 2007), characterize and evaluate the binding pattern in changed human breast tissue (Lima et al. 2010), and detect cellular changes in various tissues (Beltrão et al. 1998) distinguished by inflammation and neoplasia (Kunstfeld and Petzelbauer 2001). Lectins are important in forensic medicine for identifying brain disorders (Brinck et al. 1998) and aiding in the research and diagnosis of post-mortem human brain diseases (Brinck et al. 1998).

2.10.3 Lectin Immune Adsorbent Assay

LIA analyses IgG binding lectins using direct or indirect methods in micro titer plates with planar surfaces (solid phase), where lectin solutions passively adsorb to wells. To determine antigen antibody binding, antilectin immunoglobulin G (IgG-antilectin) coupled to peroxidase (peroxidaseantilectin-IgG) is incubated. Meanwhile, the antilectin-IgG is added and incubated in the indirect approach, followed by the addition of an IgGantiIgG-peroxidase to improve reaction specificity. In all processes, solutions containing appropriate carbohydrate for each lectin are used to avoid specific binding between the lectin and the glycidic carbohydrate part of IgG by the recognition site.

The immunoreactivity of lectins was assessed using the indirect technique LIA. For example, Cramoll 1, Cramoll 2, Cramoll 3, and Cramoll 4 isoforms from C. mollis seeds, Parkia pendula seed lectins (isoforms 1 and 2), seed lectin from P. platycephalla, commercial lectins Concanavalin A, Ulex europaeus agglutinin II (UEA-II), Triticum vulgaris lectin, Bandeiraeasimplicifolialectin II (BS-II), and Lens culinaris lectin. There are variances in carbohydrate recognition even across C. mollis isoforms from the same plant tissue. Cramoll 1, Cramoll 2, and Cramoll 4 are glucose/mannose specific, while Cramoll 3 is galactose specific. The high interaction of this lectin with peroxidase resulted in weak and inconsistent recognition among P. pendula lectin isoforms specific to glucose/mannose. A lectin structurally close to Cramoll 1 with an identity of 82% (Ulfig et al. 2004), showed limited identification; these results demonstrated the potential of LIA to assess the identity of lectins derived from various sources and species.

2.10.4 Lectin Radial Immunodiffusion and Conjugated Lectins as Tissue Markers

Radial immune diffusion revealed entire identity for Cramoll 1, Cramoll 2, and Cramoll 4, as well as a negative result for Cramoll 3; Con A lectin isoforms and lectins from P. platycephala, L. culinaris, T. vulgaris, UEA-II, and BS-II yielded negative findings. When lectins are attached to peroxidase and employed as tissue markers, a reaction product is formed that may be examined using optical microscopy. Staining intensity data from normal and transformed tissue cells demonstrate that the variations are attributable to carbohydrate alterations in tissue labeling, as determined by inhibition with lectin-specific carbohydrates.

2.11 Conclusion

Lectins are used to diagnose diseases. Lectins obtained from Freshwater and Marine organisms show their antitumor activity by inducing apoptosis and other forms of programmed cell death, inhibiting the cell cycle, and inhibiting neoangiogenesis. Lectins can also improve the antitumor activity of standard antitumor drugs. Thus, lectins' selectivity and cytotoxicity are the two main properties that elevate them to the status of ideal antitumor agents. Indeed, various synthesis strategies use genetic engineering to transfer the genes encoding the lectin of interest to microorganisms, which can be grown in large quantities, or to virus vectors, allowing the lectins to be synthesized directly inside tumor cells. Taking all of this into account, aquatic organisms are an important source of lectins, and those proteins have a promising future in anticancer research. Certainly, more research is needed to fully understand the antitumor potential of lectins in humans.

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Chapter 3
Fish Lectins: History, Types, and Structural Classification

Nayomi John, Rukhiya Salim, Swathi Ramesh, and Nivya Mariam Paul

Abstract Lectins are a remarkable class of proteins extensively found in nature. Based on their physiological properties and organization in tissues, lectins play some important roles. Lectins mediate protein–carbohydrate interactions; act as the chief constituent of innate immunity in invertebrates and vertebrates. Lectins can be useful for the analysis of structural and physiological features of cells, tissues, and harmful microbial pathogens. In agricultural sector, these lectin proteins are used as eco-friendly agents against insecticides. Lectins also play a major role from recognition of potential pathogens to complement-mediated opsonization and killing of the same by participating in several downstream effector functions. Over the last few decades, the key role of lectins as important regulators of mammalian adaptive immune responses has been identified. Fishes are bestowed with significant elements of mammalian adaptive responses and are furnished with several kinds of complex lectin repertoires. Fish lectins are highly diversified and show tissue-specific expression. The diversity of fish lectins are responsible to mediate numerous functions from cell–cell interactions, and also show some inhibitory effects against different types of bacteria, fungi, tumor cells, etc. Lectins also showed some proved biological properties like antiproliferative property of cancerous cells and mitogenic effects. Thus these proteins are hopeful drugs for the treatment of various human diseases. This review mainly focuses on the structural diversity of fish lectins and provides an overview of lectin research.

Keywords Fish lectins · Proteins · Innate immunity · Tissue specific expression · Antibacterial · Structural diversity

Abbreviations

AAA	Anguilla japonica agglutinin
ASGR	Asialoglycoprotein receptor

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CRD	Carbohydrate recognition domains
CRP	C-reactive proteins
CTL	C-type lectins
CTLD	C-type lectins domain
FTL	F-type lectins
HIV	Human immunodeficiency virus
LCDV	Lymphocystis disease virus
MAG	Myelin-associated glycoprotein
MBL	Mannose-binding lectin
MBP	Mannose-binding protein
MHC	Major histocompatibility complex
PSP	Pancreatic stone protein
RBL	Rhamnose-binding lectin

3.1 Introduction

In correlation with 90% of the biosphere, the oceans constitute about 70% of the earth's surface. Among the 30 animal phyla, 12 are exclusively marine phyla (Cheung et al. 2016). Beyond the phyla, a vast number of viruses and microbes are found. To safeguard themselves against viral and microbial attacks, marine based organisms have evolved to produce many anti-infective agents (Ogawa et al. 2011). Thus a large array of bioactive molecules acquired from marine bioresources which include fishes, invertebrate animals, bacteria, fungi, micro, and macroalgae, and other organisms.

Researchers are mainly focusing on marine natural products to produce new powerful and potential drugs including anti-HIV, anti-cancer, anti-microbials, and Alzheimer's therapeutics. Most of the bioactive compounds are organic compounds obtained from nature such as proteins, enzymes, large polymers, fatty acids, peptides, etc. Cyclic and linear peptides play a pivotal role in pharmaceutical development (Aneiros and Garateix 2004). Novel peptides from marine resources are become a new alternative for biomedical research due to their broad spectrum of activities. From natural sources, lectins are considered core of therapeutic agents in drug discovery.

3.2 Lectins

The term *lectin* meaning "selected" is a cluster of sugar-binding proteins that perceive and particularly bind to carbohydrate moieties (Cammarata et al. 2007). These non-immune origin proteins are common in nature and reversibly bind to carbohydrate moieties and glycoconjugates. Lectins are either found as free or

specifically associated with cell surfaces using particular binding sites (Santos et al. 2014). The identification takes place through specific CRDs without any change in carbohydrate residue. Lectins are mostly found in animals, plants, and bacteria. By the early 1900s, the first invertebrate lectin was uncovered in snail *Helix pomatia* (Cammarata et al. 2016). Watkins and Morgan first revealed an L-fucose-specific lectin present in a European eel species the *Anguilla Anguilla*. This in turn causes the identification of the carbohydrate feature of the H blood substance.

3.3 Animal Lectin

The lectin was first isolated from primitive marine organisms like sponges (Xiong et al. 2006). Animal lectins impart a vast progress in the area of Glyco-biology, areas of applied and basic bioscience. In some invertebrates like protozoa, insects, mollusks, crustaceans, sea cucumbers, polychaetes and sea sponges, the hemolymph and coelomic fluid are the major source of lectin (Sánchez-Salgado et al. 2014). Lectins have been recognized from various vertebrates like fish, snakes, and others. In humans, lectins are obtained from different tissues and cells, such as lungs and dendrites (Santos et al. 2014). In vertebrates, based on the location lectins are of two classes -integral lectins found in the internal part of membranes and soluble lectins present in the inter and intracellular fluids (Lakhtin et al. 2011).

3.4 Fish Lectin

Today, due to the potential of antiviral features much attention is drawn to marine lectins. Lectins are present in biological fluids like serum and mucus both extracellularly and intracellularly. Based on the CBD and requirement of divalent cations for their activities fish lectins are of the following types: C-type lectins, galectins, F-type lectins rhamnose-binding lectin, ricin-type, lily-type, and 6x β-propeller/tectonin-type lectins (Brinchmann et al. 2018). C-type lectins and F-type lectins that are mainly found in cartilaginous and bony fish are isolated from serum of fishes, mucus obtained from skin, and various other tissues (Vasta et al. 2011). Some of the lectins such as rhamnose-binding lectin (RBL) are unique to fish and have been discovered in fish eggs and embryos.

Pathogen binding lectins are an intriguing field for fish biologists. Lectins isolated from mucosal surface play a major role in immune function like agglutination, pathogen recognition, opsonization, activation of complement system, and phagocytic mechanism. The surface of fish with mucosal tissues is continuously exposed to water where abiotic factors, as well as microorganisms, viruses, and parasites, are present. Hence antibodies against mucosal lectins obtained from fish sources have been made and are considered for in vitro pathogenic studies. Other functions like protein folding, splicing of RNA, control of cell proliferation, and trafficking of

molecules are also some of the mechanisms carried out by lectins in fish. Fish lectins also have a major role in fertilization, embryogenesis, and morphogenesis. Knowledge on structural features, evolutionary, and functional characteristics of lectin immunobiology was obtained by using several fish species as model organisms (Vasta et al. 2004).

3.5 Phylogeny and Evolution of Fish Lectins

Based on the structural information regarding the protein interaction with carbohydrate domain animal lectins were initially divided into two classes like S-type and C-type. Based on the structure of the CRD, F-Type and Rhamnose-binding lectins are identified.

- **C-Type lectins:** One of the important lectin groups coming under the animal lectin families. Individuals of this group are identified by the companionship of Ca^{2+}, multiple structural domains, and carbohydrate specificity (Ogawa et al. 2011). Different members of the family that are indirectly or directly tangled in immune functions are proteoglycan core proteins, collectins, endocytic receptors, selectins, and the mannose-macrophage receptor.
- **Mannose-binding lectins:** MBL comes under the superfamily of C-Type lectins. Since MBLs are highly diversified both humans and bony fish have the same carbohydrate specificity for MBL. They are believed to be evolved before the agnathans. Throughout vertebrate evolution, they have been preserved as a marker molecule of the lectin mediated complement pathway. MBL homologs also present in some species developed from the common ancestors of both jawless and jawed fishes. Two forms of mammalian MBL are recognized; MBL A and MBL C diverged due to the separation of a common ancestor of tetrapod from bony fishes.
- **Rhamnose-binding lectin:** They are usually isolated from invertebrates, eggs, and ovarian cells of fish. RBL shows the binding activity to D-galactose. Egg lectin obtained from sea urchin is the first identified RBL family. RBLs possess variable numbers of CRDs and they are remarkably different in their length. Duplication of gene and exon shuffling leading to the divergence and evolution of RBL ancestral genes. Animal RBL CRDs were huddled into seven groups.
- **F-type lectins:** FTLs also called as fucolectins are the most structurally characterized and lately isolated lectin family in teleosts. They have been recognized in the serum of fish. Genomic databases disclose that the FTL sequence motif is phylogenetically prevalently disseminated, being present in both ecdysozoa protostomes and lophotrochozoans (i.e., mollusks and planarias) and, in a cartilaginous vertebrate, in echinoderm, and both lobe-finned, early-branching clades of vertebrates, and ray-finned fish. F-lectins are normally responsible for the regulation of immune defenses found in the bloodstream and the intestinal mucus. They are usually present in eggs, larval, and juvenile tissue components. The

absence of FTL CRDs in higher vertebrates indicates an evolutionary mystery that coexists with land colonization after cleidoic egg appearance.

3.6 Heterogeneity

Lectins have been identified from tissue components of various fish species including electric eel, conger eel, gibel carp, Japanese eel, blue catfish, catfish, channel catfish, pike perch, zebrafish, Arabian Gulf catfish, cobia fish, toxic moray, powan, Japanese trout, steelhead trout, salmons, chinook salmon rainbow smelt, bighead carp, tilapia, grass carp, ayu, mackerel, gilthead bream, blue gourami, and sea lamprey. The lectins from serum and mucus possess multiple structural and functional isoforms. Although the factors responsible for the generation of multiple isoforms are unclear, it is believed that several factors can contribute to this heterogeneity (Russell and Lumsden 2005).

In fish, lectin heterogeneity was finely demonstrated in the Japanese eel fucolectin. Japanese eel fucolectin exists as seven isoforms. These isoforms are mainly present in the liver, gill, and intestine (Honda et al. 2000). Immunohistochemistry and Northern blots revealed that these lectins were constituents of gill mucus and of hepatic origin The European eel is also known as *Anguilla anguilla* showed a novel lectin fold and a CRD sequence in their fucolectin.

The C-reactive proteins in Indian major carp also known as *Labeo rohita* showed a variation in its expression to structurally different, inducible (up to fivefold) isoforms from the normal form due to their prior exposure to different metal pollutants (Sinha et al. 2001). Mannan-binding lectin in Atlantic salmon revealed the existence of multiple genes.

These multiple or single genes produce several functional and structural innate immune molecules and prevent the multiplication of microbes, thus protecting the organism.

3.7 Zebrafish: A Model Organism to Study MBL

The lectin pathway of complement system is started by the interaction of MBL with carbohydrate residues on the microbial surface. The embryo of zebrafish contains the central components essential for the activation of complement pathway. Thus the developing embryos in early stage of zebrafish possess a functional lectin-dependent system. The early developing embryos when microinjected with anti-MBL antibody resulted in precipitation of MBL along with an increase in mortality, whereas coinjection of purified rMBL and anti-MBL antibody significantly reduces antibody-induced embryonic death rate and injection of rMBL into the embryos causes the increased level of MBL in the embryo, thus leads to their increased resistance to *Aeromonas hydrophila.* Thus in the early embryonic stage of zebrafish

prior to their immune system is fully matured, the lectin pathway is already functional, protecting the growing embryos from infections caused by microbial pathogens.

3.8 Functional Aspects of Lectin in Fish Community

- It is feasible to utilize lectins to enhance human and animal health. Lectins are potential antiviral drugs that can bind to viruses. Dln1 a pattern-like protein has high affinity and specificity to gp120 of HIV and a possible antiviral agent isolated from Zebrafish.
- Galectin AJL-1 obtained from the Japanese eel is a potential biofilm inhibitor and are used in periodontitis treatment.
- There is only slight information on the mucosal lectins functions in different locations. The high diversity in lectins of teleosts is due to the duplication of whole-genome that happened before 250 million years and are more profoundly witnessed in salmonids which possess an additional set of genomes.
- Fish mucus can induce chemotaxis in bacteria. In an experiment with gilt-head sea bream (*Sparus aurata* L.), chemotacticity was shown by several strains of *Vibrio*.
- Lectins are one of the effective agglutinins in the innate immune system. Besides lectins mucus contains many immunologically important molecules and can agglutinate bacteria and non-self-erythrocytes. Various mucosal proteins synergistically work in the process of agglutination.

3.9 Structural Diversity of Fish Lectins

Lectins are one among the promising therapeutic agents from marine bioresources and have become a major area of interest over the last few decades. Lectins are proteins that have binding sites for carbohydrates present on cells or glycoconjugates (Kumar et al. 2012) and the interaction is reversible without involving any chemical changes (Goldstein and Poretz 2012). Lectins are non-immune proteins, structurally similar to immunoglobulins, and can agglutinate cells. They are different from immunoglobulins by the difference in the composition of amino acid, use of metals, molecular weight, and its three-dimensional structure (Santos et al. 2014). The specificity of lectins to carbohydrate moieties present on the cell surface depends on the occurrence of monosaccharides or oligosaccharides that inhibit lectin-associated reactions.

Lectins are mostly divalent or multivalent proteins that reversibly interact with carbohydrate moieties on glycoproteins present either in solution or attached to cell membranes. Lectins are oligomers with Carbohydrate Recognition Domain (CRD) and possess conserved amino acid sequence within the CRD (Vasta et al. 2011). In

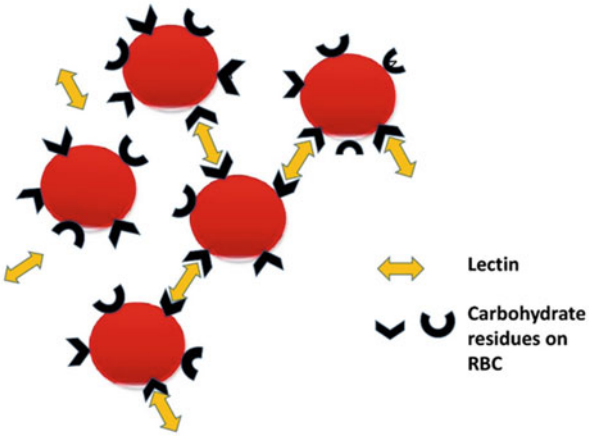

Fig. 3.1 Hemagglutination assay for lectin detection

some lectin families, the polypeptide chain includes multiple CRD's or they can be linked to other functional domains. Thus, lectins show many biological activities due to structural diversity. Isoforms within a single lectin family show differences in their specificity to the ligand they bind. The vast diversity in ligand binding is not due to genetic recombination but is imprinted in their germline (Pancer and Cooper 2006). The genetic reason for the diversification of lectin ligands is a great area of interest and it includes the development of multigene families, allelic variations, formation of complex structures by exon shuffling, and alternate splicing (Vasta et al. 2011).

Lectins mostly show high affinity towards oligosaccharides than simple sugars and structurally diverse lectins can bind to the same sugar (Peumans and Van Damme 1998). The three-dimensional structure of the binding site exhibits a conserved sequence of amino acids within the same family of lectins (Santos et al. 2014). The binding site accompanies metal ions which are mandatory to maintain the integrity of subunits (Sharon 1993). Lectins combine with carbohydrates using H-bond and hydrophobic interactions (Sharon and Lis 2002). The primary structure of a lectin is highly different from its three-dimensional structures in the number of subunits, amino acid sequence, and the number of polypeptides. The stability of lectin is decided by the interaction between the subunits. The presence of ions is another factor that determines the stability of the interaction between carbohydrates and lectins and the concentration of ions helps in micelle formation in hydrophobic sites of lectins (Santos et al. 2014).

Lectins are detected easily using agglutination assays. The specificity of lectins is easily demonstrated by hemagglutination assay using erythrocytes from different organisms (Fig. 3.1). Nattectin is a non-glycosylated C-type lectin isolated from the Brazilian venomous fish *Thalassophryne nattereri*, which exhibits hemagglutination activity independent of Ca^{2+} (Lopes-Ferreira et al. 2011). L-Rhamnose-binding lectins obtained from *Oncorhynchus keta* showed different hemagglutination patterns in rabbit and human erythrocytes (Shiina et al. 2002). Lectins are detected by

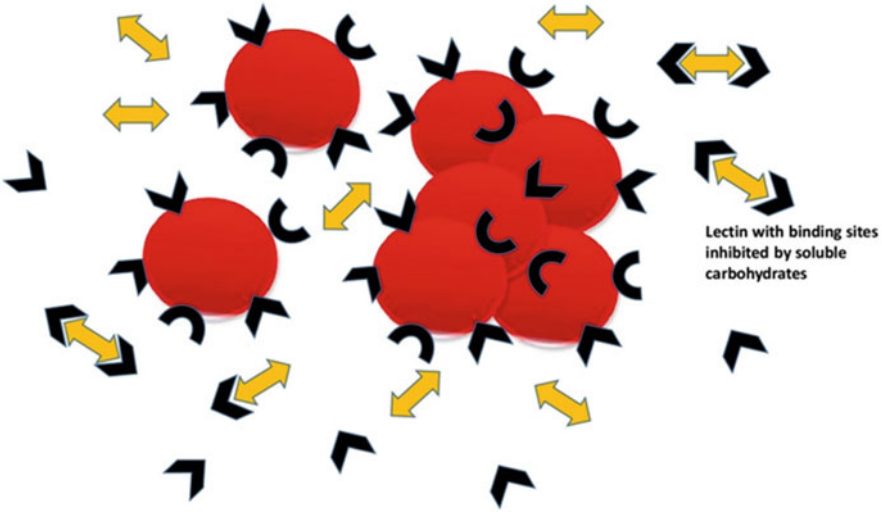

Fig. 3.2 Inhibition of hemagglutination by soluble lectins present in the medium

the inhibition of hemagglutination reaction in the presence of different carbohydrates (Fig. 3.2). Hemagglutination studies using Lily-type lectin-1 (CsLTL-1) from stripedurrel, *Channa striatus* occurred in a calcium-independent way and this property is inhibited in the presence of D-mannose and D-glucose (Arasu et al. 2013). The carbohydrate-binding ability of lectins from biological samples is also detected using an enzyme-linked adsorbent assay in which various capturing agents made of monosaccharide polyacrylamide conjugates are used (Wang et al. 2009).

Functional aspects of lectins can be demonstrated using genetic engineering in model organisms (Wang et al. 2009). Bio-informatic tools can be used for the identification of lectin domains on complex mosaic proteins. Studies on *Danio rerio* and *Takifugu rubripes* genomes revealed that they possess different galectin types and such discoveries of diverse lectins in a single individual lead to ideal techniques to analyze carbohydrate specificities of different proteins (Wang et al. 2009). Lectin specificity to numerous carbohydrate ligands can be compared at the same time using "Glyco-chips" or glycan microarrays (Comelli et al. 2006). Kinetic analysis of lectin carbohydrate interaction can be quantified using Frontal Affinity Chromatography (FAC) and surface plasmon resonance (Nakamura-Tsuruta et al. 2006; Smith et al. 2003). A recent software "LectomeXplore," used for the identification of lectins, as bioinformatics tools are nonreliable due to the small size of the functional domain and very low-level sequence similarity between carbohydrate-binding proteins.

The acquired immunity shows a specific response to infectious pathogens whereas innate immunity is more generalized with strong and vigorous responses. Teleost fish and elasmobranchs have the greatest immune response. Through serological approaches, the presence of lectin in plasma and eggs was evident in teleost

fish. Comprehensive structural and functional characterization of the fish lectin repertoires is available due to the combined applications of biochemical, molecular approaches (Vasta et al. 2004).

Lectin structure was determined by crystallization and homology modeling. For homology modeling, lectin structures from other species were used as a template. Recent studies on teleost fish also revealed the presence of efficient lectin families. These lectins are also found in other vertebrates and invertebrate taxa. This diversity in occurrence is greatly expanded by the presence of isoforms having different sugar and recognition ability.

3.10 Types and Structural Classification of Fish Lectins

Fish lectins are extensively distributed on skin mucus and immune cells associated with host defenses like renal interstitium, Hepatic sinusoids, branchial epithelium, and circulating granulocytes (Lopes-Ferreira et al. 2011). Based on the location of lectins, they are divided into soluble and integral lectins. Soluble lectins are present in intra- or inter-cellular interstitial fluids and integral lectins are present on membranes to maintain the structural identity and differ in their affinity towards different carbohydrates (Barondes 1984). In accordance with the structural diversity of CRD, fish lectins are grouped into C- type lectins (CTLs), F- type lectins, Galectins, Rhamnose-binding lectin (RBL), Lily-type, 6x β-propeller/Tectonin-type lectins (Ogawa et al. 2011).

3.10.1 C-Type Lectin Family

C-Type lectins are one of the most important animal lectins. These lectins bind to monosaccharides and oligosaccharides in the presence of calcium. They possess multiple domains which consist of 18 highly conserved residues and 14 invariant residues over many CRD's with 115–130 amino acids (Drickamer 1993). The C-type CRD consist of one or more calcium-binding sites which can even bind to carbohydrates (Ewart et al. 2001). C-Type lectin domain (CTLD) shows a loop in loop structure stabilized by two disulfide bonds and the loop region is involved in ligand binding (Lopes-Ferreira et al. 2011). Various biological processes regulated by C-Type lectins are pathogen neutralization, adhesion, and endocytosis (Ogawa et al. 2011). The CTLD superfamily consists of extracellular proteins that perform a different function but some don't exhibit lectin activity. CTLD superfamily has been divided into 17 groups after being revised many times (Ogawa et al. 2011). Group I contain a single CTLD near the C-terminus with a proteoglycan core peptide. Group II is a transmembrane protein with a carboxyl terminus in the extracellular portion and amino-terminal in the cytoplasmic side. Group III proteins are involved in innate

Table 3.1 C-type lectin groups

CTLD superfamily groups	Examples
Group I	Versican, aggrecan, neurocan, brevican
Group II	Hepatocyte asialoglycoprotein receptor (ASGR), dendritic cell intracellular adhesion molecule-3-grabbing non-integrin (DC-SIGN)
Group III-Collectins	Mannose-binding protein (MBP), proteins in pulmonary surfactant
Group IV-Selectins	E-selectin (endothelial cells), L-selectin (leucocytes), and P-selectin (platelets)
Group V	Natural killer cells, IgE receptors
Group VI	Dendritic cell surface molecule DEC-205 and macrophage cell surface mannose receptor
Group VII	Pancreatic stone protein (PSP), lithostathine

immune defense mechanisms and are composed of type I collagen strands in the amino-terminal and CTLD in the carboxyl-terminal. Group IV and V are involved in adhesive interactions and signal transduction, respectively. Group VI are transmembrane proteins with a fibronectin type-II domain, a cysteine-rich domain, and tandem CRDs. Group VII has a single CRD and no associated domains present. Major examples of each group with its functions are listed in Table 3.1.

C-type lectin proteins from fishes like *Osmerus mordax* and herring *Clupea harengus harengus* are identified to be type II antifreeze proteins (Ewart et al. 2001). C-Type lectin AJL-2 is lactose binding lectins from mucus extract of the Japanese eel *Anguilla japonica.* AJL-2 acts as major part of host defense by agglutinating *Escherichia coli* in a Ca^{2+} independent manner (Tasumi et al. 2002). LvLectin-1 and LvLectin-2 are C-type lectins purified from *Litopenaeus vannamei* are associated with the immune responses against bacteria and viruses and both require calcium ions for the carbohydrate-binding activity. Nattectin, C-type galactose specific lectin from the venom of *Thalassophryne nattereri*, involved in its innate immunity responses (Wei et al. 2012).

3.10.2 Galectin Family

Galectins or S-type lectin family consist of lectin molecules specific to galactose. Galectins are intracellular or extracellular proteins that lack disulfide bonds and carbohydrate-binding activity is independent of the presence of Calcium (Ewart et al. 2001). They possess 130 aminoacid residues within CRD and only some of the residues are involved in glycan binding. Only eight invariant residues bind to carbohydrates and the next 12 residues are highly conserved (Cummings and Liu 2009). According to their structure galectins are grouped into three types (Fig. 3.3). The prototype includes galectins with a single carbohydrate-binding domain either

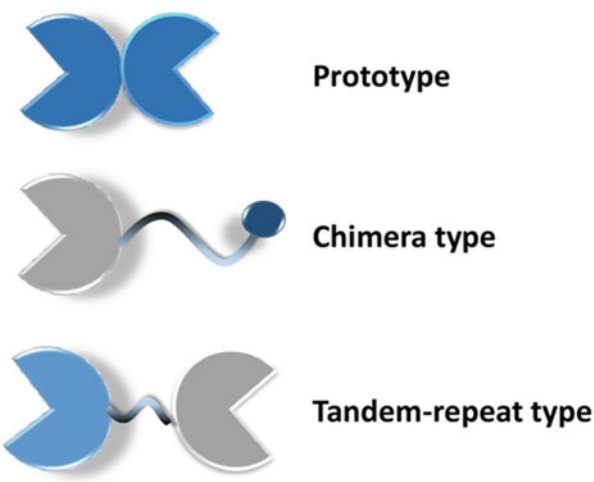

Fig. 3.3 Types of Galectins based on structural diversity

homodimer or monomer. Tandem repeats consist of two ligands binding domain on a single chain and Chimera type possess an extra N-terminal domain with the carbohydrate domain on a single chain (Ogawa et al. 2011). Galectins play a major role in diverse biological processes such as development, immunity, morphogenesis, metastasis of malignant cells, apoptosis, and many. AJL-I is a galectin from *Anguilla japonica* specific to β-galactose (Tasumi et al. 2002). Galectin-1 from *Paralichthys olivaceus* shows antiviral activity against Lymphocystis Disease Virus (LCDV) (Houzelstein et al. 2004). Three major galectin types (proto, chimera, and tandem-repeat) are found in teleost fish (Vasta et al. 2004).

3.10.3 F-Type Lectin Family

F-Type lectin or Fucolectins are non-glycosylated and show a high affinity to fucose. F-type lectin family includes diverse proteins with multiple F-type motifs, tandemly arrayed or seen in concert with other domains (Vasta et al. 2011). F-lectin, *Anguilla japonica* agglutinin (AAA) show single CRD and MsaFBP32 from *Morone saxatilis* has tandem type CRD (Ogawa et al. 2011). The CRD domain of AAA shows high similarity to different bacterial and vertebrate proteins (Bianchet et al. 2002). Trachylectin-4 from Horseshoe crab *Tachypleus tridentatus* show more strong hemagglutinating activity against human A-type erythrocytes (Saito et al. 1997). F-type lectin CRDs are absent in higher vertebrates like mammals, birds, and reptiles.

3.10.4 Rhamnose-Binding Lectin Family

Rhamnose-Binding Lectin (RBL) Family were isolated from the ovary, eggs, and skin mucus of fishes and show high affinity towards L-rhamnose or D-galactose. RBL was first isolated from eggs of sea urchin *Anthocidaris crassispina* and named Sea Urchin Egg Lectin (SEUL) (Tateno 2010). SEUL has a single carbohydrate-binding domain and exists as a homodimer, made of two identical subunits linked with a disulfide bond and it composed of 105 amino acid residues. They show hemagglutinating activity with bivalent binding properties. RBL's show tissue-specific expression. STL-1, STL-2, and STL-3 are three RBL's from *Oncorhynchus mykiss.* STL-1 protein was localized both in the ovary and tissues of the immune system but STL-2 and STL-3 were restricted only to the ovary (Tateno et al. 2002). Three RBLs from chum salmon *Onchorhynchus keta*, CSL-1, CSL-2, and CSL-3 showed different hemagglutination activity towards rabbit and human erythrocytes (Shiina et al. 2002).

RBLs are made of two or three tandem repeated CRDs, with 95 amino acid residues and eight conserved cysteine residues in each CRD. The N-terminal domain of CRD has a conserved sequence of Tyr-Gly-Arg and the C-terminal with Asp-Pro and Lys. They possess four disulfide bonds that interconnect the backbone of each domain, Cys(1)–Cys(3), Cys(2)–Cys(8), Cys(4)–Cys(7), and Cys(5)-Cys(6) (Ogawa et al. 2011). Based on the structure of the protein, RBLs are classified into three types: proto, tandem, and chimera (Fig. 3.4). The prototype consists of a single CRD and shows hemagglutination activity due to the covalent linkage via an extra cysteine residue present on the N-terminal of CRD. All RBLs from fish eggs are tandem repeat-type, two or three tandemly repeating CRDs on the same polypeptide chain. Chimera type is composed of both RBL CRD and non-RBL CRD (Tateno 2010).

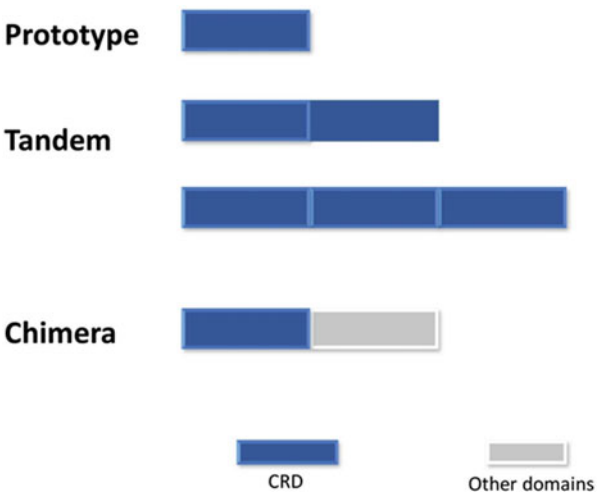

Fig. 3.4 Types of Rhamnose-Binding Lectin based on structural diversity

3.10.5 X-Type Lectins

The X-type lectins or interactions were first discovered in *Xenopus laevis* oocyte cortical granules. Interactions are glycosylated, calcium-dependent, soluble oligomer with a CRD resembling fibrinogen-like domain-specific to α-galactosides (Vasta et al. 2011). XL-35 from liver cells of rainbow trout and gcIntL from head, kidney, brain, and spleen cells of grass carp are examples of intelectins from fish (Vasta et al. 2011).

3.10.6 Lily-Type Lectin

CsLTL-1 is a lily-type lectin isolated from gills of *Channa striatus*, striped murrel, when infected with fungus and bacteria. Lily-type lectins are calcium-dependent lectins with a high affinity to mannose and glucose. The protein contains 77% coils and 23% β-sheets. The mannose-binding sites exhibit β-prism architecture and are of 30–99 amino acid residues long (Arasu et al. 2013).

3.10.7 I-Type Lectins

Immunoglobulin type lectins or sialic acid-binding immunoglobulin type lectins (Siglecs) are lectins that have CRD similar to the structure of immunoglobulins. They are known to mediate cell–cell interactions by binding to specific sialylated glycoproteins. Siglecs are found as integral proteins on the plasma membrane. Siglec1, CD22, myelin-associated glycoprotein (MAG), and Siglec15 are the four Siglecs isolated from fish (Bornhöfft et al. 2020).

3.10.8 Pentraxins

Pentraxins consists of numerous subunits with one CRD per subunit and the size of each CRD varies between 20 and 25 KDa. They are disc-shaped pentamers, which include both C-Reactive proteins (CRP) and serum amyloid p (SAP) (Vasta et al. 2011). They show calcium-dependent binding activity to carbohydrates present on bacterial surfaces which indicate their importance in host defense mechanisms (Cardoso da Silva et al. 2019). CRP I and CRP II are two pentraxins isolated from Atlantic cod (*Gadus morhua*) serum infected with *Aeromonas salmonicida.* Membrane-associated CRP shows affinity to phosphorylcholine and CRP present in the extracellular matrix towards phosphoethanolamine. Gene expression of short Zebrafish pentraxins (orthologs to mammalian C-Reactive Proteins) increased

rapidly within 5 days in virally infected fish, suggesting its role as a biomarker of viral infections in humans (Bello-Perez et al. 2021). Pentraxin from snapper *Pagrus auratus* showed opsonin activity (Cook et al. 2005).

3.10.9 Calnexin and Calreticulin

Calreticulin and Calnexin are intracellular lectins present in the endoplasmic reticulum involved in the proper folding of glycoproteins (Cardoso da Silva et al. 2019). CRD of Calreticulin and Calnexin bind to Glc1Man9GlcNAc2 of glycoproteins and possess a high affinity Calcium-binding site and globular domain for Zinc binding. Calnexin, CNX was first characterized and reported from *Ictalurus punctatus*, and determined its role in association with class II MHC (Major Histocompatibility Complex) (Fuller et al. 2004). Class II MHC α-chain of catfish lack N-linked oligosaccharide consensus glycosylation sequences. Calreticulin and Calnexin bind to different target proteins (Vasta et al. 2011).

3.10.10 Pufflectins

Pufflectins are non-covalently associated with Mannose-binding lectins identified from the skin mucus and intestine of pufferfish, *Fugu rubripes.* The pufflectin gene is expressed in oral cavity walls, gills, esophagus, and skin and an isoform only expressed in the intestine. They show 30% identity to mannose-specific lectins obtained from plant resources (Tsutsui et al. 2003).

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Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 4
Localization and Diverse Distribution of Fish Lectins

J. C. Anjana

Abstract The source of lectins derived from the marine environment is found both intracellularly and extracellularly in biological fluids such as serum and mucus. The lectins that are found to play an important role in defense mechanisms are present in both invertebrates and vertebrates such as fish. The localization and diversification of the fish lectins are mainly dependent on their gene expression or evolution. The lectin is localized in the stomach, gill, intestine, egg, serum, and plasma of different families of fish apart from the mucosal layer of fish. Lectins play an important role in the cell regulation via glycoconjugates and they specify their part in host–pathogen interactions and cell–cell communications because they identify proteoglycans, glycoproteins, and glycolipids which are special carbohydrate structures. Lectins exist in the frontier organs and tissues that demarcate the body from the outer environment and they are present in various parts such as the epidermal club cells of the skin, wall of the oral cavity, pharynx, esophagus, and gills where they become the foremost part of the biological defense system. This review deals with the localization and distribution of lectins in various body parts of the fish.

Keywords Defense · Glycoconjugates · Lectins · Localization · Mucous

Abbreviations

AFP	Antifreeze proteins
CRD	Carbohydrate recognition domain
CTL	C-type lectins
CTLD	C-type lectin-like domain
ER	Endoplasmic reticulum
EST	Expressed sequence tag
FTL	Fucose type lectin

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GANL	Homomultimeric glycolipoprotein
gcIntL	Grass carp intelectin
LPS	Lipopolysaccharides
ORF	Open Reading Frame
RbFEL	Rock bream fish-egg lectin
RBL	Lectin binding to L-rhamnose
RTLL	Rainbow trout ladder lectins
SsLec	Sebastes schlegelii lectin
YGR	Yeast Glutathione Reductase
Zfel	Zebrafish fish-egg lectin
zITLN	Zebrafish intelectin 1

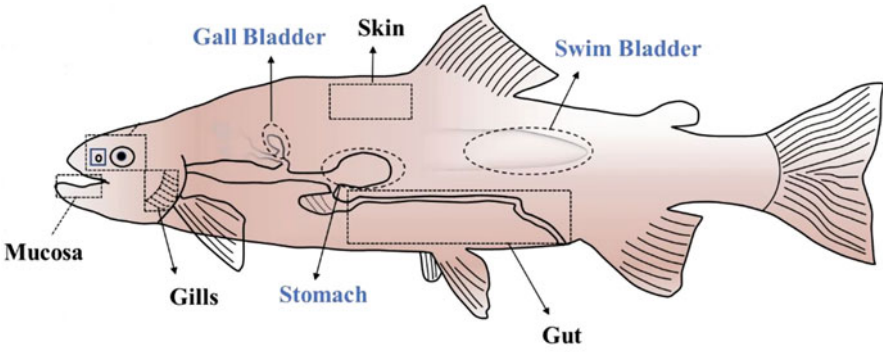
4.1 Introduction

Lectins separate certain carbohydrate structures because they are a group of proteins that bind to sugar leading to the decree of numerous cells by glycoconjugates. Peumans and Van Damme (1995), reported that lectins shows binding of non-display surface to monosaccharides or certain oligosaccharides. Lectins are involved in numerous cellular processes that rely on their recognition of complex carbohydrates that are widely distributed throughout the taxa from living organisms, plants, and animals. Natural compounds are divided into animal lectins, mushroom lectins, and plant lectins. Although lectin yield is much lower in the case of animals and mushrooms compared to plant lectins. According to reports of Sharon (2008) and Varki et al. (2009), animal lectins led to the development of various areas of biological science. C-lectins (CTLs), galectins, I-type lectins, pentraxins, and P-type lectins are some of the animal lectins that are categorized into numerous categories, depending on the similarity of the carbohydrate (CRD) recognition domain and its characteristics.

4.2 Localization of Lectins

In the spinal cord, there are two categories of lectins, based on their location: the lining membranes of the membranes and the soluble lectins present in the internal and internal fluids. The binding lectins are those found in membranes as structural elements and are particularly distinct from carbohydrates and their physical and chemical assets. Soluble lectins can move spontaneously within and between cells. In vertebrates, a group of lectin-bound cells seems to be elaborate in binding glycoconjugates to parts of the cell or vesicles, leading to the formation of glycoconjugates in certain areas (endocytosis) or similar translocation. In the meantime, the phase of soluble lectins travels to the cells that contain inside and between

cells, mixing with other soluble substances and binding membrane glycoconjugates. The fact that these proteins appear to be initially embedded within cells, and secreted by them, suggests that they have the same function of binding glycoconjugates present in and around cells (Barondes 1984).



Lectins Present in Various Parts of Fish

4.2.1 Localization of Lectins in Mucosal Surfaces

Chemicals present in water affect the eyelids, nose, mouth, intestinal tract, and bladder of fish similar to mammals and insects with epithelial cells throughout the mucous membranes. The risk arises from the constant exposure of mucosal tissue to the surface of fish in the water where there are viruses, microorganisms, and parasites, as well as abiotic factors. Therefore, the chemical habitats of fish must have a strong immune system to maintain homeostasis and health and protect against the dangers of pollution. The surface of the mucosal skin is the largest area in the fish. In recent years - omics technology has established a knowledge base related to the molecules present in the skin (Brinchmann 2016) and the teleostal mucosal surface (Salinas and Magadan 2017).

According to Nickel (2003) and Chua et al. (2012), lectins present within the mucous membrane are found internally and externally. Lectin concealment and transport are done by old and unusual means of transport. One method involves the classical endoplasmic reticulum (ER)/Golgi apparatus secretory, in which the protein is made by ER translation and eventually sent to the cell surface. Non-traditional transport of lectins includes alternatives such as channels or carriers, exosomes, and microvesicles. Generally, glycoproteins are secreted proteins in the ER-Golgi pathway and carbohydrates are added to the ER and Golgi via N-linked glycosylation and O-linked glycosylation, respectively. Pufflectin (mannose-binding lectin), derived from the skin of pufferfish as reported in Fugu rubripes, appears to be non-glycosylated, as reported by Tsutsui et al. (2003). Natalin is found without cells

in the skin, seen by Patel and Brinchmann (2017) and Rajan et al. (2017), is suggesting a non-traditional method of concealment (Rajan et al. 2017). Galectins are also lectins that exhibit an unusual secretion mode (Nickel 2003; Cooper and Barondes 1990).

Studies of skin mucus lectins have been performed on many species of fish including windowpane flounder *Lophopsetta maculata* (Kamiya and Shimizu 1980), Arabian Gulf catfish *Arius thalassinus* (Al-Hassan et al. 1986), dragonet *Repomucenus richardsonii* (Shiomi et al. 1990), and kingklip *Genypterus capensis* (Toda et al. 1996). Among these lectins, the main structures were determined by the only conger eel of galectins called congerins I and II (Muramoto and Kamiya 1992; Muramoto et al. 1999). Yuasa et al. (1998) reported that the sequence structure of lectin skin other than fish was limited and reported in only two species: the land slug *Incilaria fruhstorferi* and the African frog with *Xenopus laevis* (Marschal et al. 1992). Hemagglutinating activity caused by the presence of lectin was reported in the sticky skin of the Japanese eel *Anguilla japonica* by Suzuki (1985). According to Suzuki and Kaneko (1986), the lectin found in club cells (eel epidermis) is composed of epithelial cells and mucous cells. Tasumi et al. (2004), reported that galectin AJL-1 from the Japanese eel is found in the skin and mucous membranes, and the substance is expressed only in the skin.

4.2.2 Localization of Lectins in Other Organs

Lectins can be deposited in the cell, cytosol, and within cells. According to Rajan et al. (2013), antibodies fight against the two homologs Codgal1 -1 and Codgal1 -2 in Atlantic cod (*Gadus morhua*) and are labeled separately in the skin, cavity, and intestines. Codgal1–1 is found in the epithelial layer in the skin, cavities, and rectum, while the closest homologous Codgal1–2 is found in epithelial cells and basal cells in the skin such as Codgal1–1. Lectin was isolated from the cobia's ovaries *(Rachycentron canadum*) which exhibits antibacterial activity against Escherichia coli as observed by Ngai and Ng (2007). Galectin from Atlantic cod is also found in the skin mucus (Rajan et al. 2011). About seven fucolectins were found in the European eel (*Anguilla anguilla*), where two continued to be studied and showed differences in the distribution. According to a study by Honda et al. (2000), one substance was introduced in the liver to release proteins that were translated into serum, while another was expressed in the gut and may have played a role in mucosal protection. Therefore, several lectin homologs may be present in a particular species. The species exhibits a variety of tissue and cell processing due to the presence of several homologous lectins, and in addition, differential expression can be found at different stages of fish development.

4.2.2.1 Lectins in Gills

Lectins such as FTL and GANL have an apparent weight of 220 kDa and were isolated from the gills of bighead carp. They had activity against *Vibrio harveyi* but showed negligible anti-fungal activity (Pan et al. 2010). Six RBLs (IpRBL1aAQ13, IpRBL1b, IpRBL1c, IpRBL3a, IpRBL3b, and IpRBL5a) found in channel catfish contained one to three CRD with well-preserved YGR and DPC markers as reported by Thongda et al. (2014).

4.2.2.2 Roe Lectins

The lectins are highly present in fish eggs. According to work reported by Kim et al. (2011), increased manifestations of lectin were observed at 1, 3, 12, 24, and 36 h after fertilization. One such lectin is zFEL, separated from zebrafish that resembles a maternal substance capable of increasing the phagocytic activity of macrophages that fight Gram positive and Gram negative bacteria (Yousif et al. 1995; Wang et al. 2016). In the typical Japanese catfish (*S. asotus*) Murayama et al. (1997) reported about a 95 kDa molecular weight roe lectin, SAL. Approximately three galectin galectins (Drgal1-L1, Drgal1-L2, and Drgal1- L3) have been identified and identified in zebrafish playing an important role in the early kingdom (Ahmed et al. 2004) .

4.2.2.3 Lectins from Kidney

The kidney is another significant tissue where lectin is expressed. FTL RbFTL-1 was identified in rock bream with full-length cDNA composed at 1204 bp with an open reading framework of 945-bp (ORF) containing 314 amino-acid proteins (Park et al. 2012). In a recent study of Wang et al. (2016) he had explained tandem double galectin-9, PfGAL9 from large yellow catfish, which contained two distinct CRDs with two high-binding sugar motifs. These lectins have been shown to play a key role in the natural response to bacterial infections by eliminating them. At the same time, Zhang et al. (2016) identified a new galectin-9, LcGal9 in a large yellow croaker exposed to the kidneys, liver, spleen, intestines, and lungs.

4.2.2.4 Lectins from Liver

According to studies by Lee et al. (2016), it has been shown that lectins 2 and 3 are more abundant in the liver and skin and are produced by body-related tissues when the tissues are fully formed. In addition, the expression of CTL, SsLec1 has been proven to occur mainly in the liver of blackfish (*Sebastes schlegelii*). These lectins exhibit stored CRD sequences, mannose-binding motifs, calcium-binding sites, and four disulfide bond structures that form cysteine residues (Liu et al. 2016).

4.2.2.5 Lectins from Serum

Dutta et al. (2005) separated lectin-binding lectin and lectin-dependent pH with a molecular weight of approximately 200 kDa from the serum of Indian catfish (*Clarias batrachus*). Lectin showed the specificity of a-methyl galactose and sialoglycoproteins (Table 4.1).

4.3 Diverse Distribution of Lectins

Lectins are different molecules by structure (Shirai et al. 2009); this structural complexity, which is associated with these proteins, reflects a large number of families. Russell and Lumsden (2005) and Lin et al. (2009) reported that lectin formation is important in defining the characteristics of glycan classes found in several species and currently, animal lectins are classified, grouped by shared evolutionary origin and/or similarity of structural folds. S-lectins are classified as thiol-dependent proteins for intra- and extracellular localization mainly in response to β-galactosides as identified by Drickamer (1988). Three major types of galectin, proto, chimera, and tandem-repeat, are present in telb6bgeost fish. The Galectin-3 sequence was determined from the pufferfish genome *Tetraodon nigroviridis* (AL301540) and the zebrafish EST *Danio rerio* (BM034940) was used in comparative studies with human galectin-3 (HSPC159) (Cooper 2002).

4.3.1 Diverse Tissue-Specific Expression of C-Type Lectins

According to Vijayan and Chandra (1999), C-type domains are commonly found in animal lectins from serum, extracellular matrix, and membranes. C-lectins have been identified in various species of fish (Ourth et al. 2008), Japanese flounder *Paralichthys olivaceus* (Kondo et al. 2007), the poisonous fish *Thalassophryne natterere* (Lopes-Ferreira et al. 2011). Calnexin and calreticulin are related proteins that represent the group of intracellular lectins, endoplasmic reticulum proteins that interact less with glycoproteins and may contribute to the threat but may work to keep proteins properly integrated into the endoplasmic reticulum. They have a lectin site that detects oligosaccharide processing between folding glycoprotein, Glc1Man9GlcNAc2 (Williams 2006). They have been identified in mammals, plants, in fish salmonids such as rainbow trout, *O. mykiss*, cyprinids by Kales et al. (2004, 2007) and Bielek (2008). Lectins of the large Immunoglobulin family (Siglecs) are a subgroup formed by the formation of lectins of type I. They are compound membrane proteins, expressed specifically in the plasma membrane (Angata and Brinkman Van der Linden 2002).

Table 4.1 Source of fish lectins and their tissue-specific expression (localization of lectin)

S. no.	Lectin name	Fish source	Lectin family	Tissue localization	Reference
1.	OfLTL-2 and 3	Rock bream (*Oplegnathus fasciatus*)	Lily type lectin	Liver and skin	Lee et al. (2016)
2.	RbFEL	Rock bream (*Oplegnathus fasciatus*)	Fish-egg lectin	Liver and head kidney	Kim et al. (2013)
3.	Kalliklectin (40 kDa)	Fugu (*Takifugu rubripes*)	C-type lectin		Tsutsui et al. (2015)
4.	LcGal9	Yellow croaker (*Larimichthys crocea*)	Galectin	Liver, spleen, kidney, head kidney and intestine	Zhang et al. (2016)
5.	Tn pufflectin	Puffer fish (*Takifugu niphobles*)	C-type lectin	Skin	Tasumi et al. (2016)
6.	Lyc CTLR	Yellow croaker (*Larimichthys crocea*)	C-type lectin	Liver and heart	Ao et al. (2015)
7.	SauFBP32	Atlantic salmon (*Salmo salar*)	C-type lectin	Serum	Ewart et al. (1999)
8.	KPL	Skipjack tuna (*Katsuwonus pelamis*)	C-type lectin	Hard roe	Jung et al. (2003)
9.	Congerin	Conger eel (*Conger myriaster)*	Galectin	Skin mucus	Nakamura et al. (2012)
10.	AAA	European eel (*Anguilla anguilla*)	F-type lectin	Serum	Bianchet et al. (2002)
11.	RcaL	Cobia fish (*Rachycentron canadum*)	C-type lectin	Serum	Coriolano et al. (2012a, b)
12.	HjCL	Japanese bullhead shark (*Heterodontus japonicus*)	C-type lectin	Skin	Tsutsui et al. (2015)
13.	PfGAL9	Yellow catfish (*Pelteo bagrus*)	Galectin	Head, kidney, trunk kidney, liver, spleen and blood	Wang et al. (2016)
14.	BGL	Pallus (*Trichogaster trichopterus*)	C-type lectin	Serum	Fock et al. (2001)
15.	T- antigen binding lectin	Snakehead murrel (*Channa striatus*)	C-type lectin	Plasma	Manihar and Das (1990)
16.	CBL	Indian catfish (*Clarias batrachus*)	C-type lectin	Serum	Singha et al. (2008)

(continued)

Table 4.1 (continued)

S. no.	Lectin name	Fish source	Lectin family	Tissue localization	Reference
17.	SAL	Catfish (*Silurus asotus*)	C-type lectin	Egg	Murayama et al. (1997)
18.	SaIntL	Catfish (*Silurus asotus)*	Intelectin	Kidney, skin and gills	Tsutsui et al. (2011)
19.	RBL	Channel catfish (*Ictalurus punctatus*)	RBL	Mucous	Thongda et al. (2014)
20.	AJL-2	Japanese eel (*Anguilla japonica*)	C-type lectin	Skin	Tasumi et al. (2002)
21.	eCL-1 eCL-2	Japanese eel (*Anguilla japonica*)	C-type lectin	Gills	Mistry et al. (2001)
22.	AJL-1	Japanese eel (*Anguilla japonica*)	Galectin	Skin	Tasumi et al. (2004)
23.	Variants of Drgal	Zebra fish	Galectin	Roe	Ahmed et al. (2004)
24.	GANL	Bighead carp (*Aristichthys nobilis*)	F-type lectin	Gills	Pan et al. (2010)
25.	Onil	Tilapia fish (*Oreochromis niloticus*)	C-type lectin	Serum	da Silva et al. (2012)
26.	Nattectin	Thalassophryne nattereri	C-type lectin	Venom	Lopes-Ferreira et al. (2011)

Source: Elumalai et al. (2019)

4.3.2 Diverse Tissue-Specific Expression of F-Type Lectins

According to Russell et al. (2008a, b), rainbow trout ladderlectins RTLL1 and RTLL 2 are expressed in the kidneys, intestines, bones, and skin. FTL from sea bass (*Dicentrrchus labrax*) is expressed by hepatocytes and intestinal cells, and the protein is found in plasma and intestinal tissues as reported by Salerno et al. (2009) and in eggs and larvae (Parisi et al. 2010). The pentraxins resemble family members, some of whom act as acute phase reactants in teleostrums thus rapidly increasing their plasma value in response to stress, injury or infection but for others, the most common protein pentraxins are present in normal plasma (Lund and Olafsen 1999). According to Tasumi et al. (2002), FTLs from linear bass appear to be present in large plasma levels of undisputed populations, with a moderate increase

in the production of Lipopolysaccharides (LPS), while in Japanese eel hepatocytes (A. japonica), FTL enters Infectious challenges in fish are quickly controlled by FTL, thus behaving as acute phase respondents. In these species, the expression of mucus galectin was higher in people resistant to infection (Tasumi et al. 2004). Intelectin (gcIntL) extracts are applied to the heads of the head, spleen, and intestines, as well as to proteins found in various organs and tissues in LPS carp challenge (reviewed by Vasta and Ahmed 2008).

4.3.3 Diverse Tissue-Specific Expression of Rhamnose-Binding Lectins

The novel family of rhamnose-binding lectins (RBLs) was identified in the eggs of steelhead trout (Tateno et al. 2002) and also reported by Mercia et al. (2013). Many RBLs, attached to the liver by oocytes, are still widely distributed in adult tissues including spleen, thrombocytes, and blood leukocytes. Tsutsui et al. (2003) reported that pufflectin genes are expressed through gills, oral wall, soap, and skin, as similar isoforms, with isoform expressed only in the intestines. Pufflectins are different from lectins that bind purified mannose to puffer fish plasma, and recent work has revealed that it is still widely distributed in the families of the most active fish.

4.3.4 Diverse Tissue-Specific Expression of Intelectin

Intelectins, a type of galactan binding galactan rainbow trout, are expressed only in the liver. There are different types of intelectin, where Intelectin 1 is expressed in all tissues of different carp species but the expression patterns is different in species of catfish for intelectin 2. In this case it is commonly expressed in the liver and also in minimal volume in the intestines and kidneys. In blue catfish, intelectin 2 is most commonly found in the liver, kidneys, head, and heart, and is also present in the intestines, middle kidneys, and gill (Takano et al. 2008). Grass carp intelectins are mainly expressed in the kidneys and head and are also found in the intestines and gills (Chang and Nie 2007). Intelectin in rainbow trout is expressed in the gill, liver, intestines, and skin (Russell et al. 2008a, b). According to the work reported by Chen et al. (2016, 2018, 2020) and Lin et al. (2009), the highly expressed intelectin in Zebrafish is Intelectin1 (zITLN1) which is highly expressed in the intestines, spleen, and liver. Intelectin 2 (zITLN2) and intelectin 3 (zITLN3) are present in the intestines and liver, respectively, whereasIntelectin 4, (zITLN4), and intelectin 6, (zITLN6), are not expressed at all. The intelectin5 (zITLN5) is produced in infected fish but is more prone to sputum and throat and in a very low amount in healthy zebrafish. Yan et al. (2012), had reported a similar compound in the digestive tract and on the skin of amphioxus. AmphiITLN239631 is expressed in the various

parts of zebrafish and is found in high amounts in the hepatic cecum and the lowest muscle expression. In *Ciona intestinalis*, intelectin was mainly distributed to serum (Abe et al. 1999; Satou et al. 2002).

Nakamura et al. (2009) identified the activity that binds lectin-fucose-binding to a protein similar to C1q from surfperch (*Neoditrema ransonnetii*); it is articulated in the liver, stomach, and intestines, and is raised by a bacterial challenge. Like pufflectins, the sweet homotetrameric protein (plumieribetin) from the extracts and scorpion-fish skin (*Scorpaena plumieri*) exhibits the synthetic properties shared with B-lectins that bind the mannose plant, known for its role in bacterial protection. However, plumieribetin inhibits the binding of α1β1 integrin to the lower cell IV in Ca^{2+}−independent interactions, protein–protein, weakening cell-collagen contacts, and reducing cell proliferation (de Santana et al. 2009).

4.4 Perspectives

Over the past decade, fish lectin research studies have revealed the role of these proteins in the reproductive system of these vertebrates. These proteins can bind to cells, and reduce polysaccharides, glycoproteins, or glycolipids that mediate various biological processes such as cell–cell interactions, correction of glycoproteins, apoptosis, antibacterial, and antiviral activity. Fish lectins play a major part against fish diseases and research has engrossed in the isolation of bioactive molecules and associated microbiota due to their importance in fish farming. The presence of lectins in fish plays a major role in social interactions among fish, such as commensalism, amensalism, and symbiosis. The bioactive substances obtained from fish lectin mucous have great potential in aquaculture and human medicine. They also sought to have an association with microbiota linked with it. Lectins are believed to mediate the detection of viruses in fish antibodies that perform an imperative part in the body's response.

4.5 Conclusion

The structure and composition of lectins in a comprehensive variability of fish with different and special carbohydrate properties may be effective in self-defense. Genomic studies and the writing of fish lectins will reveal the magnitude of gene expression that helps to understand its structure in various organs. The omics technologies that include genomics, transcriptomics, proteomics, and metabolomics help in the study of various genes that have a huge possibility for the extrapolation of fish lectins in detail with their role in several biotic events. A strenuous demonstration of the mechanisms by which each lectin or its isoforms play their role in various environmental functions will be of great interest to researchers and is of great importance to industry and society.

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Part II
Crustacean and Molluscs Lectins

Chapter 5
Investigation on Mollusc Lectins

M. Chellapackialakshmi and C. Ravi

Abstract Mollusca is one of the largest phyla in the animal kingdom that inhabit diverse ecosystems across the planet earth. The innate immunity of mollusk plays a significant role in recognizing and eliminating pathogen as it lacks antibody mediated immunity. Pattern recognition receptors (PRRs) in the cytoplasm of molluscs recognize conserved structures of different pathogen associated molecule patterns (PAMPs) which is vital for pathogen physiology and pathogen fitness. One of the domains in PRRs is lectin which is a carbohydrate binding protein that acts as a defense molecule against pathogens, excludes them out and protects the animal. Mollusc has different types of lectins such as C-type, P-type, F-type, I-type, S-type (Galectins), Ficolins and Chitinase like lectins which have greater applications as therapeutic agent to treat cancer, bacterial, and fungal diseases. Mollusc lectins are involved in host recognition and tissue adhesion and also in drug discovery process. Most of the researchers found that C-type lectins play a key role in immune recognition and defense mechanism.

Keywords Lectin · Pattern recognition receptors (PRRs) · Pathogen associated molecule patterns (PAMPs)

Abbreviations

ADEL	*Aplysia dactylomela* egg lectin
AK	Arginine kinase
C3	Complement component 3
CGL	*Crenomytilus grayanus* lectin
CRD	Carbohydrate recognition domain
CTL	C-type lectins
DlFBL	*Dicentrarchus labrax* fucose binding lectin
FBG	Fibrinogen like domain

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FREPS	Fibrinogen related proteins
HddSBL	*Haliotis discus discus* Sialic acid binding lectin
Ig	Immunoglobulin
LPS	Lipopolysaccharide
LRR	Leucine rich repeat
LTA	Lipoteichoic acid
M6P	Mannose-6-phosphate
MAC	Membrane attack complex
MAN	Mannan
MCL	*Mytilus californianus* lectin
PAMPs	Pathogen associated molecule patterns
PEPCK	Phosphoenol pyuruvate carboxy kinase
PGN	Peptidoglycan
PRRs	Pattern recognition receptors
SABL	Sialicacid binding lectins
SR	Scavenger receptor
TLRs	Toll like receptors

5.1 Introduction

Molluscs are bilaterally symmetrical, soft bodied, triploblastic, and eucoelomate invertebrates that inhabit diverse niches across the globe. Animals belonging to phylum Mollusca usually have a shell with a mantle, ventral foot, anterior head, and a dorsal visceral mass. There are more than 50,000 known molluscan species that include the snail, clams, oysters, octopus, and the squids (Hickman et al. 2002). They are important ecosystem engineers that play a significant role in structuring the ecosystem, thereby provide food, shelter, and protection to a wide range of animals (Fortunato 2015). Mollucs have been a source of food for human beings since time immemorial and humans used them in making jewels, tools, and lime.

The vast majority of molluscs inhabit the sea where they live in hostile intertidal rocky shores and benthic region and are continuously exposed to physical and chemical stress, susceptible to injury, and microbial attacks. They have the immune system without immunological memory but the innate immunity recognizes pathogens and eliminate them by various processes. Hence, molluscs have the ability to adapt themselves and overcome those obstacles by the synthesis of various secondary metabolites (Pati et al. 2015). Marine molluscs are exposed to an important selective pressure called as biofouling and this pressure has led to the evolution of bioactive compounds against predation and surface fouling (Srikumaran et al. 2011). The potential of marine mollusc as source of biologically active products is largely unexplored in India and large scale screening of bioactive compounds from mollusc is mandatory (Sivasubramanian et al. 2011). Natural products have a diverse range of therapeutic applications that include antimicrobial, antioxidant, anticoagulant,

anti-inflammatory, anticancer, wound healing, and immune modulator properties (Mayer et al. 2013).

5.2 Immune Defense in Molluscs

Molluscs have the inherent ability to exert comparatively good immune response against foreign particles and organisms. The innate immunity of molluscs imitates the inherent non-specific response that provides the first line of defense (Khalaifah and Nasser 2018). The main elements of innate immune response are physical barriers, phagocytic cells, and physiological components. The cellular components are the circulating hemocytes which invades small pathogens and eliminate them by phagocytosis, while large pathogens are eliminated by encapsulation. Humoral immune components includes nitric oxide, lysozyme activity, lectins, and the phenyloxidase system.

Hard shells of molluscs act as barriers to prevent pathogens from penetrating into the host's body. Mollusc shells contain organominerals especially calcium carbonate and their compositions depends on the species. Most of the shell has three layers (periostracum, prismatic layer, and nacreous layer). These layers of the mollusk are compactly packed and their acidic environments prevent the growth of microbes (Khalaifah and Nasser 2018). The mucous membrane present in the tissues and tracts of soft bodied mollusc conflict with pathogenic microbes and inactivate the pathogen by encapsulation by hemocytes. As a result, there is a movement of the outer cells in the body which expels out trapped pathogens.

The first line of immune defense of molluscs against pathogens is hemocytes which are equivalent to leukocytes in higher animals. It plays a major role in innate immune defense action by engulfing extracellular particles by phagocytosis, encapsulation, and endocytosis. These cells are also involved in digestion, excretion, healing of wounds, shell repair, and transport of nutrients. Bivalve hemocytes produce positive chemoattraction towards products released by infectious agents ranging from trematodes to various bacterial species (Allam and Raftos 2015). Other than hemocytes, some of the soluble physiological elements such aslysosomal activity, lectins, phenyloxidase system and nitric oxide defends and eliminate foreign pathogens by innate immune responses. In bivalves, lysozyme activity is to protect them from microbial invasions, and it will increase as soon as the pathogen entered into the body (Allam and Raftos 2015).

5.2.1 Complement System

Complement components are proteins circulating in the blood (inactive form). When antigen is present, complement gets activated and enter into biochemical cascade which helps to eliminate antigens by lysis of cells, opsonization, and binding to

Table 5.1 Different types of domains in the pattern recognition receptors (PRRs) of Mollusc

S. no	Pattern recognition domain
1.	C1q domain
2.	Ig domain
3.	Lectin domain
4.	Leucine rich repeat (LRR) domain
5.	Scavenger receptor domain (SR)

Table 5.2 Proteins involved in pattern recognition receptors of the defense mechanism

S. no	Proteins in PRRs
1.	Arginine kinase (AK)
2.	C1q domain containing proteins (C1qDCs)
3.	Fibrinogen related proteins (FREPS)
4.	Lipopolysaccharide (LPS)
5.	Lipoteichoic acid (LTA)
6.	Mannan (MAN)
7.	Petidoglycan (PGN)
8.	Phosphoenol pyruvate carboxy kinase (PEPCK)
9.	Scavenger receptors (SRs)
10.	Toll like receptors (TLRs)

specific complement receptors on cells of the immune system (Khalaifah and Nasser 2018). The mannan binding lectin pathways are an innate immune response but antibody independent pathway. When foreign substances enter into the body of mollusc, complement component 3 (C3 complement) gets activated. Lectin binds to a mannose carbohydrate on the cell wall of pathogens such as *Salmonella* and the mannose binding lectin pathway gets activated. Production of lectin (serum acute phase protein) from the inflammatory response in the site of inflammation and membrane attack complex (MAC) is produced upon activation of the complement system.

Pattern recognition receptors (PRRs) in the cytoplasm of molluscs recognize conserved structures of different pathogen associated molecule patterns (PAMPs) which is essential for pathogen physiology and pathogen fitness (Wang et al. 2018) and the different types of domains in PRRs are presented in Table 5.1. The involvement of proteins in the pattern recognition and the subsequent immune response has been presented in Table 5.2 and Fig. 5.1.

5.3 Lectins

Lectins are carbohydrate binding protein that acts as defense molecule against foreign cells and exclude the pathogens including bacteria, yeast, and multi-cellular parasites such as trematodes. Diversely, lectins are found in viruses, bacteria, fungi, plants, invertebrates, and vertebrates (Allam et al. 2020). Lectins are also present in mucous covering gills and labial palps. Lectin and carbohydrate combination

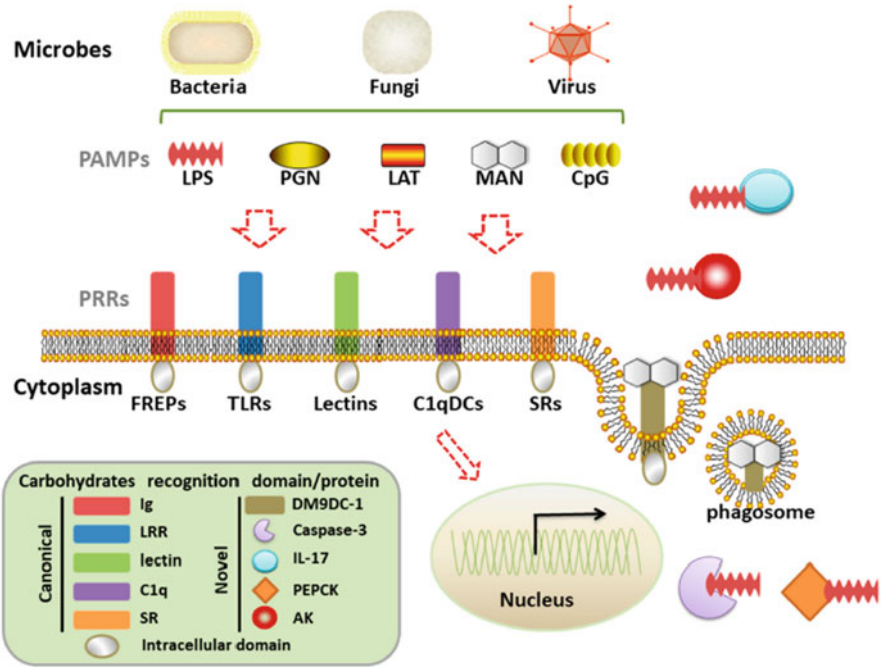

Fig. 5.1 Mollusc Immune defense mechanisms by carbohydraterecognition proteins (Wang et al. 2018). Pattern recognition receptors (PRRs) in the cytoplasm of mollusc (FREPs, TLRs, Lectin, C1qDCs, SRs) recognizes Pathogen associated molecule pattern (PAMPs) (LPS, PGN, LAT, MAN, CpG) of various pathogen such as bacteria, fungi and virus; exclude them out by phagocytosis process

represent ligand and receptor interaction. Binding capacity of lectins is based on its structure and affinity to molecules like glycoproteins and glycolipids. The specificity of lectins is analyzed based on hapten inhibition test in that various sugars will be tested for their capacity to inhibit hemagglutination of erythrocytes (Kumar et al. 2015). The first lectin called as ricin was isolated from extracts of *Ricinus communis* and another lectin called as abrin was obtained from extracts of *Abrus precatorius* which have the ability to agglutinate blood cells (Santos et al. 2014).

5.4 Chemical Nature of Lectins

Lectins are oligomeric proteins of diversified structure and molecular size that differ in primary, secondary, and tertiary structure, number of subunits and subunit assembly, metal requirement, as well as in the constitution of carbohydrate binding sites (Sharon and Lis 2013). The polypeptide chains comprise various molecular domains among them, carbohydrate recognition domain (CRD) plays a key role in

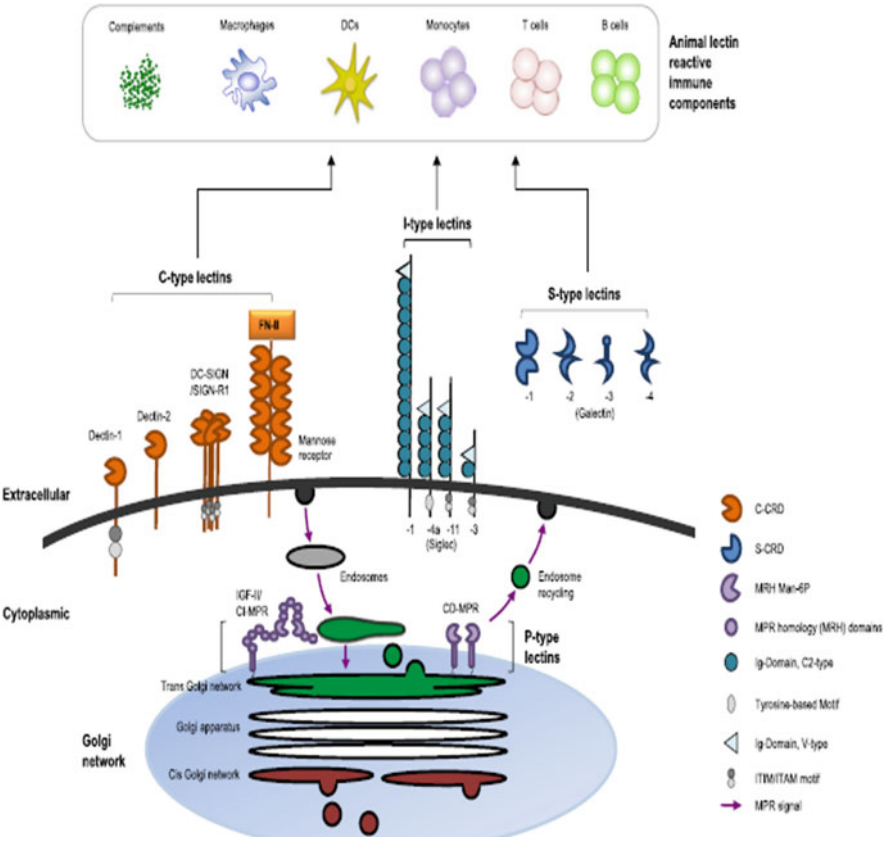

Fig. 5.2 Structure and location of different types of animal lectin (Loh et al. 2015). Display of Animal lectin (C-type, S-type and I-type) and its reactive immune components (Macrophages, Monocytes, T cells and B cells)

recognition of pathogens and it is present only in animal lectin (Varrot et al. 2011). Lectins combine with their ligands by hydrogen bonds and hydrophobic interactions (rarely), electrostatic interactions, and coordination with metal ions. These bonding were initiated by water molecules. Other than CRD, C-terminal fibrinogen like domain (FBG) which is polymorphic in vertebrate immune system and emerging as important immune factors of the innate immune response of invertebrates (Khalaifah and Nasser 2018). The location of different types of lectins are depicted in Fig. 5.2.

5.4.1 *Purpose of Lectin Production and Its Significance*

Lectin production has been proved as an innate immune response against infection and facilitates cell to cell contact. These are non-enzymatic and non-immunoglobulin carbohydrate binding proteins which reversibly bind to specific carbohydrate structures, either free in solution or on cell surfaces (Singh et al. 1999). Comparatively, research found that an increasing number of lectins do not show high affinity to simple saccharides (Khalaifah and Nasser 2018). Lectins play major role in the process of nonself recognition against pathogens, agglutination, opsonization, phagocytosis, predation, symbiosis, capture, and ingestion of microalga in invertebrates (Wang et al. 2013). In molluscs, lectins are often involved in host recognition and tissue adhesion and also in internal biological process such as reproduction. It has the ability to agglutinate cells through interaction with carbohydrates present on the cell surface. Hemocyte activation by phagocytosis process triggers the production of cytokines and also releases antimicrobial compounds (Allam and Raftos 2015).

5.5 Application of Lectins

Animal lectins are involved in cell trafficking, immune regulation, and prevention of autoimmunity (Loh et al. 2015). Involvement of lectins was analyzed in membrane structures of cells, glycosylation pathways, differentiation, cell division, growth and developmental changes, and mapping neuronal connections (Khalaifah and Nasser 2018). Nowadays, the potential of lectins as therapeutic agents against cancer, HIV, and Alzheimer diseases has been analyzed and included in the drug discovery process (Ogawa et al. 2011). Lectins are vital contributors to tumor cell recognition (surface markers), cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, augmentation of host immune defense, cytotoxicity, and apoptosis (Hamid et al. 2013). Lectins are useful as histochemical and cytochemical reagents to examine changes that occur in the cell surfaces during physiological and pathological processes, from differentiation to cancer (Sharon and Lis 2013).

Marine lectins include galectin from *Anguilla japonica* lectin 1, *Haliotis discus discus* sialic acid binding lectin (HddSBL), *Dicentrarchus labrax* fucose binding lectin (DlFBL), and *Strongylocentrotus purpuratus* rhamnose binding lectin used to treat various cancer and leads various cancer cells' death by apoptosis (Wu et al. 2017). Lectins are also associated with the transport of sugar and carbohydrate storage (Kumar et al. 2015). Interestingly, lectins have been used in research for the identification and interaction of microalgal species such as symbiosis (Espinosa et al. 2009). Expression of mucosal lectins (CvML, CvML3914, and CvML 3912) in *Crassostrea virginica* was analyzed and it regulates reproduction process (Allam et al. 2020). C-type lectins can recognize diverse bacterial pathogens and induce

cytokine production and they are also involved in clearance, homeostasis, and immunomodulation (Loh et al. 2015).

Galectin-1 initiates cell adhesion and apoptosis, and regulates cellular proliferation; Galectin-3 initiates cell adhesion, regulates inflammation, pre-mRNA splicing, and protects against induced apoptosis. Galectin-4 and galectin-6 have an important function in cell–cell and cell–extracellular matrix crosslinking; galectin-5 is involved in maturation and erythrocyte adhesion (Loh et al. 2015).

5.6 Lectins in Mollusc

Animal lectins are carbohydrate binding proteins ubiquitously found in all organisms which vary in their functions, structures, tissue localizations, and carbohydrate binding specificities and it has its own CRD with identical sequences and consisting of four cysteine residues and two disulfide bonds (Loh et al. 2015).

Invertebrate lectins seem to be highly conserved in sugar binding ability. Therefore in mollusc, lectins from different species display diversified sugar specifities such as *Limax flavus* is specific for sialic acid; *Helix pomatia* for c-N-acetyl D-galacosamine; *Achatinina fulica* for O-acetyl-sialic acid; and *Tridacna maxima* for galactose (Espinosa and Lozano 1997).

In bivalve mollusc, lectins are present in blood cell (hemocyte) or hemolymph or free in plasma and act against the invading pathogens (Allam et al. 2020). Agglutinating activity of lectin was detected in eggs and glands of reproductive tract or mucous of different molluscan sp. Among 13 lectin families in animals, seven types of lectins has been identified in molluscs such as: C-type, P-type, F-type, I-type, S-type (Galectins), Ficolins, and Chitinase like lectins, respectively.

5.6.1 C-Type Lectins (CTL)

C-type lectins (Calcium dependent lectins) have double loop structure where the second loop is long; involved in Ca^{2+} dependent carbohydrate binding and consist of two conserved disulfide bridges situated at the base of loops. Presence of calcium ions activates surface bound recognition molecules. These lectins are endocytic receptors and its expression is seen in macrophages and some endothelial cells (Loh et al. 2015). It is multivalent in nature with 18 conserved amino acid residues. Its main role is to identify and bind to terminal sugars on PAMPs and participate in immune recognition against pathogens. Most of the mollusc lectin has single CRD, while multi-CRD lectins have been identified from *Argopecten irradians* which has greater affinity to bind with Lipopolysaccharide (LPS) from Gram negative bacteria; Petidoglycan (PGN) from Gram positive bacteria and β-glucan and Mannan from fungi (Wang et al. 2018). More than 30 CTLs has been identified in mollucs (Table 5.3).

Table 5.3 C-type lectins in Mollusc

Mollusc	C-type lectin (CTL)	Carbohydrate recognition domain (CRD)
Japanese little neck (*Venerupis philippinarum*)	1	VpCTL
Japanese abalone (*Haliotis discus hannai*)	2	CLHd and HdhCTL1
Traingle sail mussel (*Hyriopsis cumingii*)	2	Hcperlucin and HcLec4
Manila clam (*Ruditapes philippinarum*)	2	MCL-3 and MCL-4
Farrer's scallop (*Chlamys farreri*)	6	CfLec-1-CfLec-4, CfLec-4b, CfLec-5
Oyster (*Chlamys gigas*)	7	CgcLec-1-CgcLec-7
Bay scallop (*Argopecten irradians*)	10	AicTL-1-AicTL-10

5.6.2 P-Type Lectins

P-type lectins are intracellular transmembrane glycoproteins which are specific to mannose-6-phosphate (M6P) to identify lysosomal enzymes that requires Ca^{2+} for activity in the trans Golgi network and deliver to prelysosomal compartments (Loh et al. 2015).

5.6.3 F-Type and (Fucolectins/Fucose Binding Lectins) and I-Type Lectins

F-type lectins specifically binds to fucose molecule and act as molluscs recognition in both vertebrates and invertebrates. F-type of lectins is a carbohydrate recognizing protein which belongs to Immunoglobulin (Ig) super family. It is a large group of proteins which contains 70 to 110 amino acids compactly arranged with two planes of α pleated sheet. The stacked J-sheets are folded with intrachain disulfide bridge between two conserved Cysteine residues. These lectins recognize sialic acids which is acidic monosaccharides present at the outer end of secreted cell surface of glycoconjugates. More than 40 different forms of sialic acid exist in a variety of linkages to sugar (Angata and Linden 2002). Sialic acid binding lectins (SABL) has affinity towards N-acetyl nuraminic acid (NeuNAc), N-acetyl-D-galactosamine (GalNAc), N-acetyl D-glucosamine (GlcNAc), N-acetyl-D-mannosamine (ManNAc) (Ghosh 2017).

Table 5.4 Involvement of multiple CRD of galectins and its immune response against pathogens

Multiple CRD	Organism	Immune response
CvGal	*C. virginica*	Microbial pathogens, unicellular algae, *Perkinsus trophozoites*
PoGal-1 and PoGal-2	*P. fucata*	Bacteria
AiGal-1 and AiGal-2	*A. irradians*	*E. coli*, *V. anguillarum, M. luteus, V. fluvalis, Edwardsiella tarda*
MCGal	*R. phillippinarum*	*Perkinsus olseni* and agglutinate *V. tapetis* in vitro

5.6.4 S-Type Lectins (Galectins)

Galectins are conserved and ubiquitous family found in metazoan organisms. It is cation independent binds specifically to β-galactosidase. Based on its structure, galectins can be categorized into prototype (monomer or homodimer of single carbohydrate binding domain), tandem repeat type (two carbohydrate binding domains on a single chain), and chimera type (carbohydrate binding domain and an extra N-terminal domain on a single chain). Some of the galectins have one CRD, while others have clustering of multiple CRDs. Especially, in mollusc, Quadruple CRDs galectins including AiGal-1 and AiGal-2 has been reported from *Crassostrea virginica* and *Pinctada fucata* (Wang et al. 2013). C-type lectin, D-galactose binding lectin, anti-B like agglutinin and scalarin were identified in egg moss fluid of different molluscs like snail (*Biomphalaria glabrata*, *Pomaceasclaris*, *Pila ovate* and sea hare *Aplysia kurodai*) (Wang et al. 2014). The involvement of multiple CRDs of galectins and its immune response against various pathogens has been presented in Table 5.4.

5.7 Significance and Application of Mollusk Lectins

Molluscan C-type lectins have agglutinating property of various microbes as well as vertebrate erythrocytes (Wang et al. 2011). Bivalves have antiviral effector and antibacterial activities by producing proteins such as histones and lysozymes and it has been reported in clams and *C. gigas* (Allam and Raftos 2015). The lectins produced by mollusc play a significant role in protecting the animal by exerting immunity (Table 5.5) and its biological activities has been explored further (Table 5.6) by the biomedical industry.

Table 5.5 Types of lectins involved in mollusc and its significance

S. no	Lectin type	Name of lectin	Organism	Significance	Reference
1.	C-type	AiCTL	*Argopectens irradians*	Inhibit the microbial growth, cellular adhesion	Huang et al. (2014)
		Cflec-1 and Cflec-2	*Chlamys farreri*	Phagocytosis, encapsulation	Yang et al. (2011)
		–	Manila clam (*Ruditapes philippinarum*)	Antibacterial activity	Takahashi et al. (2008)
		–	Oyster (*Crassostrea virginica*)	Anifungal activity	Chikalovetes et al. (2015)
2.	F-type	PmF	Oyster (*Pinctada martensii*) and *C. gigas*	Immune response	Wang et al. (2018)
3.	I-type (SABL)	–	*Solen grandis* and *Limax flavus*	Bacterial recognition	Ghosh (2017)
		9-O-AcSA	Snails (*Achatina fulica*)	Innate immunity, bacteriostatic effect	Biswas et al. (2000)
		SgSABL-1 and SgSABL-2	*Solen grandis*	Pathogen recognition	Yang et al. (2012)
		Cgsiglec-1	Oyster (*Crassostrea gigas*)	Innate immunity	Wang et al. (2018)
		Ch-salectin	*Crassostrea hongkongensis*	Bactericidal activity	He et al. (2011)
		VpSABL	*Venerupis philippinarum*	Recognition of bacteria (− strain)	Li et al. (2011)
		MCsialec	*Ruditapes philippinarum*	Antibacterial activity	Adhya et al. (2010)
4.	Galectin	–	Sea mussel (*Crenomytilus grayanus*)	Antibacterial activity	Kovalchuk et al. (2013)
		PoGal-1 and PoGal-2	*P. fucata*	Immune response against bacteria	Wang et al. (2018)
		CvGal	*C. virginica*	Phagocytosis	Wang et al. (2018)
		MCL	*M. californianus*	Antibacterial activity	Wang et al. (2018)
		GalNAc	*Helix pomatia*	Protect from bacterial invassion	Wang et al. (2014)
5.	Chitinase like lectins	Cgclp1 and Cgclp2	*Crassostreag igas*	Innate immunity	Badariotti et al. (2006)
6.	Ficolins	chFCN	*Crassostrea hongkongensis*	Phagocytosis	Xiang et al. (2014)
7.	H-type	HcLec4	*H. cumingii*	Antibacterial activity	Wang et al. (2018)

Table 5.6 Application of mollusc lectins

S. no	Lectin type	Significance	Application
1.	C-type	Cell adhesion, glycoprotein clearance, Innate immunity, recognition of pathogens, endocytosis	Phagocytosis, encapsulation, antifungal, and antibacterial activity
2.	P-type	Endocytosis, lysosome biogenesis, protein sorting post golgi, glycoprotein trafficking, ER associated degradation of glycoproteins, enzyme targeting	Generation of functional lysosomes, signal transduction
3.	F-type	Innate immunity	Immune response
4.	I-type	Cell adhesion, regulation of myeloid cell interaction, signaling	Bacterial recognition
5.	Galectins	Glycan cross linking in the extracellular matrix	Antibacterial activity
6.	Ficolins	Innate immunity	Phagocytosis
7.	Chitinase like lectins	Collagen metabolism	Innate immunity
8.	H-type lectins	Diagnosis of diseases, involve in drug delivery systems	Treatment for breast and colon cancer

5.8 Research Trends in Lectins

Novel Galactose binding lectins (MCL) isolated from *Mytilus californianus* (García-Maldonado et al. 2017) and (CGL) from Sea mussel (*Crenomytilus grayanus*) exhibit antibacterial activity against Gram positive and Gram negative bacteria (Kovalchuk et al. 2013). Novel lectin from *Aplysia dactylomela* eggs (ADEL) was separated by affinity chromatography which agglutinates and inhibits biofilm formation of *Staphylococcus aureus* and potentially used as antimicrobial agents (Wang et al. 2018). Recently "omics" technology (Genomics, Proteomics, Transcriptomics, and Metabolomics) provides the resources for understanding the mechanisms and effectors of bivalve immunity. To date, the complete genome information of six molluscs such as pearl oyster *Pinctada fucata*, the Pacific oyster *Crassostrea gigas*, the Mediterranean mussel *Mytilus galloprovincialis*, the owl limpet *Lottia gigantea*, the snail *Biomphalaria glabrata*, and the Californiasea hare *Aplysia californica* has been revealed. This number is expected to increase in the upcoming years (Allam and Raftos 2015).

Mollusc lectin especially C-type lectins participate in immune recognition and defense against pathogens to exclude them out. Lectins also play various roles as drug to treat cancer and bacterial and fungal diseases. Lectins from mollusc have a great potential to serve as future therapeutic agents and more focused research is required in this area.

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Chapter 6
Investigation of Lectins from Anomuran and Brachyuran Crabs

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Abstract Lectins are a class of specific carbohydrate binding proteins that can agglutinate cells or precipitate polysaccharides and glycoproteins and function as recognition molecules. They vary in molecular size, amino acid composition, three-dimensional structure, metal requirement, and function in order to adapt to the challenges of the antigens/ pathogens in the surrounding environment. There are different types of lectins such as C-type (calcium dependent lectin), S-type or Galectins (β-galactoside binding lectins), L-type (lectins containing leguminous lectin domain), MBL (mannose-binding lectin), P-type (mannose 6-phosphate receptor specific lectin), N-type (recognize mannose 6-phosphate N-glycans), R-type (contain structurally similar CRD to ricin), Collectins (collagen-containing C-type lectins), I-type (immunoglobulin type lectin), etc. Siglecs are a group of I-type lectins with sialic acid (Sia)-specificity and an amino-terminal. Such sialic acid specific lectins are of growing interest as reagents in biochemical research and diagnostic analysis and can be extremely useful to study sialic acids in diagnosis of pathogenic disease and tumors. The prevalence of such impressive sialic acid specific lectin with significant biomedical prospective is reported among arthropods and molluscs. Among these, the lectins of crabs were studied elaborately and almost all reported crab lectins, especially brachyuran crabs are of sialic acid specific. This chapter deliberates the lectins identified from anomuran and brachyuran crabs.

Keywords Agglutinin · Arthropods · Crabs · Crustacea · Hermit crabs · Lectin · Malacostraca · Sialic acid · Siglecs

Abbreviations

AgNPs	Silver nanoparticles
AiL	*Atergatis integerrimus* lectin
AMP	Adenosine monophosphate

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Ara	Arabinose
BSM	Bovine submaxillary mucin
cDNA	Complementary DNA
CFL	*Charybdis feriatus* lectin
CNBr	Cyanogen Bromide
ConA	Concanavalin A
CRD	Carbohydrate-recognition domain
CTL	C-type lectin
EDTA	Ethylene diamine tetra acetate
EIL	*Erimacrus isenbeckii* lectin
EPS	Exopolysaccharide
ESI-Q-TOF-MS	Electrospray ionization quadrupole time-of-flight mass spectrometry
EsLecF	*Eriocheir sinensis* lectin
ESM	Equine submaxillary mucin
EST	Expressed sequence tags
ETA-1	*Episesarma tetragonum* agglutinin 1
ETA-2	*Episesarma tetragonum* agglutinin 2
FITC	Fluorescein isothiocyanate
FPLC	Fast performance liquid chromatography
FTIR	Fourier transform infrared spectroscopy
Gal	Galactose
GalNAc	N-acetyl galactosamine
GlcNAc/GluNAc	N-acetyl glucosamine
HA	Hemagglutination
kDa	Kilo Dalton
Lac	Lactose
LTLs	L-type lectins
MALDI-TOF	Matrix assisted laser desorptional/Ionization-time of flight
ManNAc	N-Acetyl mannosamine
Meli	Melibiose
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NeuAc	N-acetyl neuraminic acid
NeuGc	N-glycolyl neuraminic acid
OSM	Ovine submaxillary mucin
Pjlec	*Paratelphusa jacquemontii* lectin
PPA	*Philyra pisum* agglutinin
PPL	*Philyra pisum* lectin
Pp-Lec	*Portunus pelagicus* lectin
ProPO	Prophenoloxidase
PRR	Pattern recognition receptor
PSM	Porcine stomach mucin
PtCLec2	*Portunus trituberculatus* C-type lectin 2

PtCTL1	*Portunus trituberculatus* C-type lectin 1
PtLTL	*Portunus trituberculatus* L–type lectin
RBC	Red blood corpuscles
rEsLecF	Recombinant *Eriocheir sinensis* lectin
rPtCTL1	Recombinant *Portunus trituberculatus* C-type lectin 1
rSpCTL-B	Recombinant *Scylla paramamosain C-type lectin*
SDS-PAGE	Sodium dodecyl sulphate- poly acrylamide gel electrophoresis
SpCTL-B	*Scylla paramamosain C-type lectin*
SSAP	A protein *Scylla serrata* lectin
WSSV	White spot syndrome virus

6.1 Introduction

Arthropods, a successful group occupying all possible habitats such as deep sea, estuary, freshwater, desert, underground, and on other organisms as parasites form the largest group in the animal kingdom. They are diverse due to their well defending characters and adaptation, and play key roles in human welfare and health. Crabs are a group of invertebrates belonging to the Phylum Arthropoda and grouped under the Class Crustacea (exoskeleton impregnated with hard shell); Subclass Malacostraca, Order Decapoda, Suborder Anomura (Hermit crabs), and Brachyura (true crabs). This chapter highlights the lectin investigated in anomuran and brachyuran crabs.

Anomurans unlike the true crabs of Brachyura are adapted to live their peculiar mode of life with strange modifications. Broad cephalothorax, flattened with a hard shell makes it distinct from true crabs.

Brachyuran crabs show the highest degree of specialization. The cephalothorax of brachyurans is broader than anomurans with small feelers. Antennules and eye stalks are present in sockets of the carapace and the third maxillipede is flat. The first chelate leg has pinching claws and other legs are clawed and non-chelate (Kotpal 1969).

Crabs possess an excellent immune system to defend itself from the surrounding pathogens similar to other living organisms. The immune system in crab is of innate type comprising of both cellular and humoral immunity. The humoral response is mediated by factors such as prophenoloxidase, toll-free receptors, antimicrobial peptides, lysins, lipopolysaccharide-binding proteins, clotting factors, complement factors, protease inhibitors, agglutinins/lectins. Thus lectin/ agglutinin/ hemagglutinin is an important defense molecule, compared to the antibodies of vertebrates involved in pathogen recognition and elimination. It exhibits opsonization by enhancing/ mediating the phagocytic ability of the hemocytes. Based on the nature, lectins are grouped into many types such as C, S, L, N, P, R, I, etc. Among this, one subtype of I-type lectin is sialic acid specific lectin called siglecs, which has high

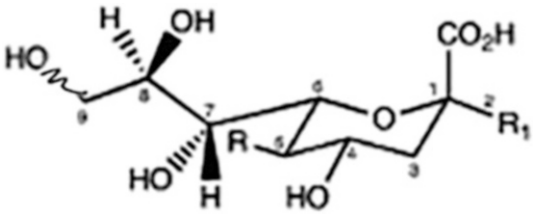

Fig. 6.1 Sialic acid

biomedical potential due to its capacity to bind specifically the sialoglycoconjugates on glycocalyx of cell surface.

Sialic acid specific lectins (siglecs) are a subset of I-type lectin, involving in cell–cell interaction and carbohydrate recognition (Angata and Van der Linden 2002). The sialic acid/ neuraminic acid specificity is one of the remarkable characters of the lectin in crabs. This specificity is highly important in biomedical research as diagnostic and therapeutic tool. The sialic acids (sias) are a family of acidic monosaccharides with nine carbon backbone, typically terminating the outer ends of cell surface glycan chains, transferred using α 2 → 3, α 2 → 6 or α 2 → 8 linkages to sub terminal sugars by a family of about 20 sialyl transferases and they serve as recognition sites (Fig. 6.1) to which pathogens attach and also perform important intrinsic function required for normal development (Sharon 2008; Varki 2007). The presence of sialic acid specific lectin (siglecs) among crabs is gaining attention owing to its biological (Fig. 6.2) and clinical applications (Fig. 6.3). Hence, investigation of lectins in crabs has captured the interest of many researchers worldwide.

6.2 Crab Lectins

Crabs rely solely on innate immunity (Huang and Ren 2020) which is based on both cellular and humoral immune response. The cellular immune mechanism includes phagocytosis (Foukas et al. 1998), encapsulation (Asgari et al. 1998), nodule formation (Koizumi et al. 1999), and autophagy (Yau et al. 2015). The humoral immune response includes clotting system (Hall et al. 1999), potent toll like receptor mediated antimicrobial proteins (Underhill and Orinsky 2002), prophenoloxidase (ProPO) activating system (Soderhall and Cerenius 1998). It is also triggered by lipopolysaccharide (Koizumi et al. 1999), β-1-3 glucans (Soderhall and Cerenius 1998), peptidoglycans (Ochiai and Ashida 1999), hemolin (Mendoza and Faye 1999), melanin formation (Sugumaran 2002), C-reactive proteins, scavenger receptors, Down syndrome cell adhesion molecules, thioester containing proteins (Huang and Ren 2020), α_2-macroglobulin and lectins (Sharon and Lis 2004). Lectins involved in cell recognition and communication, host–pathogen interaction, embryogenesis and tissue development (de Oliveira Figueiroa et al. 2017), cell adhesion and localization, signal transduction across membranes, mitogenic

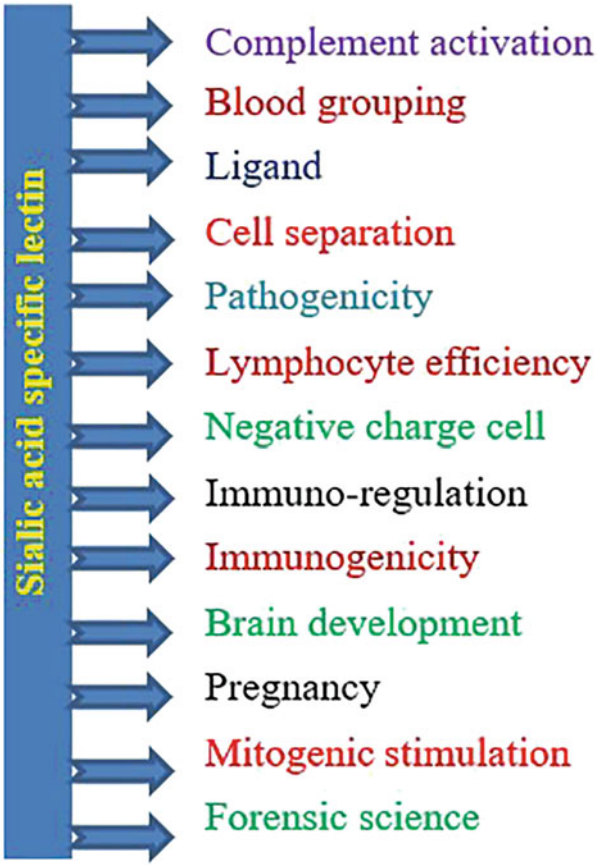

Fig. 6.2 Applications of sialic acid specific lectins (Siglecs)

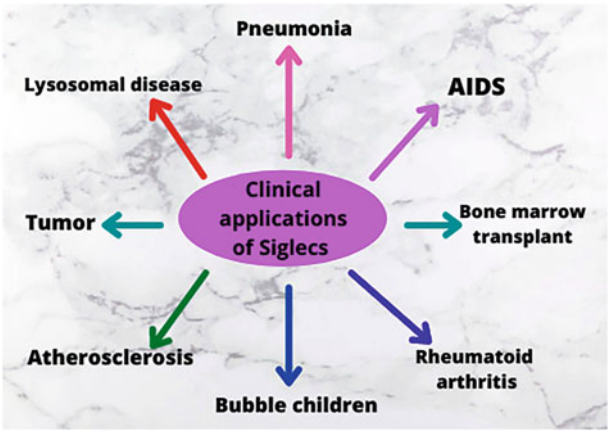

Fig. 6.3 Clinical applications of sialic acid specific lectins (Siglecs)

stimulation, augmentation of host immune defense, cytotoxicity, and apoptosis (Mody et al. 1995).

Identification, characterization, and purification of agglutinins/ lectins, a family of recognition factors from the hemolymph and other tissues of many species of brachyuran crabs have been reported. These lectins are involved in defense strategies of the crabs (Zhang et al. 2011).

Physico-chemical characterization of the crab lectins/ agglutinins revealed some common features. They are natural, proteinaceous, heat labile, pH sensitive, and calcium dependent (Ghidalia et al. 1975; Cassel et al. 1986; Umetsu et al. 1991; Chattopadhyay and Chatterjee 1993; Nalini et al. 1994; Denis and Mercy 1999; Mercy et al. 2004; Congjie et al. 2008; Jayaraj et al. 2010; Rama Devi et al. 2012; Bai and Rose 2020). However, each one had its unique carbohydrate binding specificity, such as GalNAc, GlcNAc (Umetsu et al. 1979; Cassel et al. 1986), terminal glucose with α 1–2 glucosidic linkages (Nalini et al. 1994; Jayaraj et al. 2010; Meena et al. 2011b), NeuAc (Ravindranath et al. 1985; Fragkiadakis and Stratakis 1997; Denis et al. 2003; Rama Devi et al. 2012), NeuGc (Mercy and Ravindranath 1993; Kim et al. 2006; Devi et al. 2013a), and D-mannose (Wei et al. 2018). The lectin may occur as a single lectin (Mercy and Ravindranath 1992) or multiple lectins (Cassel et al. 1986; Umetsu et al. 1979; Chattopadhyay and Chatterjee 1993; Devi et al. 2013a). The molecular mass of crab lectins varies for each species with subunits ranging from 4.8 to 300 kDa (Ghidalia et al. 1975; Umetsu et al. 1991; Chattopadhyay and Chatterjee 1997) suggesting the presence of unique lectins in crabs. Immuno-challenge with bacterial and viral pathogens induced the production of lectins (Denis et al. 2015; Zhang et al. 2020).

Although hundreds of lectins are known, sialic acid specific lectin referred as siglecs, an I-type lectin is of growing interest due to its wide application in the field of glycobiology, cell biology, immunology, oncology, and other areas of research (Wagner 1990) as sialic acids play an important role in biological processes including malignancy (Schauer 1985). Lectins that recognize the linkages or modifications of sialic acid are important in biochemical research and diagnosis (Wearne et al. 2006) and can be immense value in biopsy of tumors and identification of pathogens (Varki 2008). The most preferred ligand of the crab lectins are the family of sugars called sialic acids, which encompass more than 18 members (Schauer 1987). Most of the agglutinins/ lectins of crabs exhibited sialic acid specificity (Ravindranath et al. 1985; Mercy and Ravindranath 1993; Denis et al. 2003; Mercy et al. 2004; Kim et al. 2006; Na et al. 2007; Bai and Rose 2020). Anticancerous property of sialic acid specific lectins have been documented from crabs *Philyra pisum* (Kim et al. 2006) and *Scylla serrata* (Pramanik et al. 2010; Surapong Pinitglang et al. 2013). The presences of sialic acid specific lectins in the crabs which are not capable of synthesizing sialic acids suggest that the lectins perform a defensive role in the immunological system of the crabs.

6.3 Investigation of Lectin in Anomuran Crabs

Phylum	Arthropoda
Class	Crustacea
Subclass	Malacostraca
Order	Decapoda
Suborder	Anomura

6.3.1 Family: Diogenidae; Genus: Diogenes

A naturally occurring hemagglutinin that exhibited high agglutination activity with rat erythrocytes was reported in hemolymph of *Diogenes affinis* (Murali et al. 1994), a hermit crab. Cross adsorption tests with different RBCs indicated that it is a single agglutinin. Ammonium sulphate precipitation of the serum agglutinin yielded a lectin of MW 185 kDa with non-identical subunits.

The agglutinability was Ca^{2+} dependent, N-acetyl hexosamine specific, sensitive to EDTA, stable between pH 6.0 and 7.5, and temperature 20 and 50 °C. In contrast, hexose as well as hexosamine counter parts exhibited no inhibitory effect, expressing the essentiality of N-acetyl group for agglutinin–ligand interaction. However, among the glycoproteins BSM and porcine thyroglobulin, BSM that contain terminal NeuAc showed inhibition and porcine thyroglobulin did not show any inhibitory effect (Schauer 1982). The acetylated amino sugars found in LPS of *Salmonella* (Luderitz et al. 1968) and other bacteria (Slifkin and Doyle 1990) specifically recognized the agglutinin of *Diogenes affinis*, indicating that the *Diogenes affinis* lectin play an important part in the host–pathogen interactions.

6.4 Lectin Investigation in Brachyuran Crabs

Phylum	Arthropoda
Class	Crustacea
Subclass	Malacostraca
Order	Decapoda
Suborder	Brachyura

6.4.1 Family: Gecarcinucidae

6.4.1.1 Genus: *Cardisoma*

The presence of agglutinins/ lectins was reported in brachyuran crabs since 1971. Smith and Goldstein (1971) identified a hemolymph agglutinin in *Cardisoma guanhumi* that specifically recognized the sperm cells of sea urchin *Tripneustes ventricosus*.

6.4.1.2 Genus: *Paratelphusa*

An agglutinin that showed the highest agglutinating activity towards the rabbit erythrocytes was observed in *Paratelphusa hydrodromous* (Nalini et al. 1994). The agglutinin of this freshwater crab was heat labile with maximum activity between pH 7.5 and 10.

An O-acetyl sialic acid specific lectin that expressed high agglutinating activity with horse and rabbit erythrocytes was detected in the hemolymph of *Paratelphusa jacquemontii* (Denis et al. 2003). The serum agglutinin activity was high between the temperature 30–35 °C and pH 7.5 and above. A 34 kDa lectin was purified using BSM coupled agarose column and eluted with EDTA. Bovine sub-maxillary mucin that contains 9-O-acetyl- and 8, 9, di-O-acetyl-N-acetyl neuraminic acid was the effective glycoprotein inhibitor. The lectin failed to agglutinate sialidase treated and de-O-acetylated rabbit erythrocytes, which confirmed the O-acetyl NeuAc specificity. The lectin of *P. jacquemontii* has the capacity to recognize the sialyl residues on the surface of the bacteria.

The opsonic function of the lectin (Pjlec) of *P. jacquemontii* was evaluated (Denis et al. 2015) through its binding specificity with the glycoconjugates/ glycosylated cell surface of pathogens. Investigation on induction revealed that high agglutinating erythrocytes induced augmentation of agglutinin activity and resulted in an increased survival rate of crabs. Faster clearance of the erythrocytes of higher agglutinability and lectin-coated erythrocytes than the erythrocytes of low agglutinability and uncoated erythrocytes revealed the biological function of this *P. jacquemontii* lectin as an opsonin in the defense strategy of the crab. Pjlec, the lectin of *Paratelphusa jacquemontii* induced the synthesis of Ig (immunoglobulin) in the serum of mice (Balb/c). Anti-Pjlec was purified by affinity chromatography using Protein A Sepharose column. Injection of Anti-Pjlec into the hemocoel of the crab enhanced phagocytosis (Denis et al. 2017).

6.4.1.3 Genus: *Barytelphusa*

The hemagglutinin activity of various tissues such as hemolymph, hemocytes, extracts of hepatopancreas, mantle, heart, muscle, gills, ovary, and anal tube of

Barytelphusa cunicularis was analyzed and maximum agglutination activity was observed in the hemolymph. Though marked difference in HA was not observed between male and female crabs, the size of the crabs showed considerable influence on HA activity. The hemolymph agglutinin was specific to rabbit erythrocytes, calcium dependent, sensitive to pH (6.5–8.0) and temperature (30–40 °C). Cross adsorption test with agglutinating erythrocytes revealed the presence of a single agglutinin (Josephine Priyatharshini et al. 2018).

6.4.1.4 Genus: *Oziotelphusa*

A natural hemagglutinin with high agglutinability towards rabbit erythrocyte and the glycoprotein fetuin and sugar α-lactose was identified in the hepatopancreas of the freshwater crab, *Oziotelphusa naga* (Vargila et al. 2020).

6.4.1.5 Genus: *Lamella*

A naturally occurring agglutinin from the serum of *Lamella lamellifrons* was purified by affinity column chromatography and biospecific adsorption (Bai and Rose 2020). The molecular weight of the lectin was identified as 68 kDa on SDS-PAGE. The agglutinin specifically agglutinated rabbit, buffalo, and rat erythrocytes. The lectin was calcium dependent with optimum HA activity at pH 7–7.5 and temperature 0–40 °C. The hemagglutinability was inhibited by glycoproteins BSM, bovine thyroglobulin, porcine thyroglobulin, fetuin, holotransferrin, lactoferrin and sugars NeuAc, lactose, glucose, fucose, melibiose, etc.

6.4.1.6 Genus: *Macropipus*

The serum agglutinin with a molecular weight of 300,000 Da electrophoretic mobility similar to human γ_1 (β_2) globulins and a specific antigenicity was purified from the crab *Macropipus puber* (Ghidalia et al. 1975).

6.4.2 Family: Coenobitidae; Genus: Birgus

A lectin, which preferentially agglutinated mouse and human A erythrocytes was identified in the serum of the coconut crab, *Birgus latro*. It showed equal reactivity with untreated, pronase treated and neuraminidase-treated erythrocytes. The serum of the crab contained distinct lectins specific for sialic acid and non-identical carbohydrate moieties (Vasta and Cohen 1984).

6.4.3 Family: Cancridae

6.4.3.1 Genus: *Cancer*

The agglutinating activity of three species of crabs, *Cancer antennarius, Cancer productus,* and *Cancer anthonyi* was investigated (Ravindranath and Cooper 1984). The agglutinin in *C. antennarius* agglutinated horse, mouse, rat, and rabbit erythrocytes. Cross reactivity experiments indicated that the erythrocytes agglutinated by serum agglutinin shared a common receptor but with a qualitative difference. The agglutination of horse erythrocytes was inhibited by BSM but not by fetuin, thyroglobulin, acid glycoprotein, PSM or colominic acid. The lectin was purified using BSM-agarose affinity column and showed a MW of 70 kDa on SDS gel electrophoresis. BSM containing 9-O-acetyl and 8, 9 di-O-acetyl sialic acid, equine submaxillary mucin (ESM) having 4-O-acetyl sialic acid and ovine submaxillary mucin (OSM) possessing N-acetyl neuraminic acid greatly inhibited the hemagglutinability. The O-acetyl sialic acid specificity was confirmed by the loss of hemagglutination inhibition of BSM on desialylation and de-O-acetylation (Ravindranath et al. 1985).

6.4.3.2 Genus: *Callinectes*

The hemolymph of blue crab, *Callinectes sapidus* was assessed for the presence of hemagglutinin. The serum and hemocyte microsomal fractions of the blue crab, *Callinectes sapidus* contained heterogeneous lectin (Cassel et al. 1986). Cassel et al. (1994) found that hemolymph lectins of *C. sapidus* recognized selected serotypes of its pathogen, *Vibrio parahaemolyticus*. Lectins agglutinated both untreated and enzyme treated erythrocytes and lymphoid cell lines. N-acyl amino sugars like sialic, N-acetyl muramic, and N-acetyl glutamic acids, GalNAc, GluNAc, and ManNAc and glycoproteins BSM, human orosomucoid, PSM, and colominic acid inhibited the serum and hemocyte lectins at low concentrations. The serum of this crab contained multiple lectins that recognized the pathogenic strains and mediated their clearance (Vasta 1992). A natural hemagglutinin highly specific for rabbit erythrocyte was identified in the serum of the swimming crab *Callinectes danae* (Moura et al. 2015) averaging 5 cm in length.

6.4.4 Family: Portunidae

6.4.4.1 Genus: *Charybdis*

Anti-B agglutinins with specificity for human B, rabbit, and guinea pig erythrocytes were reported in the hemolymph of the swimming crab, *Charybdis japonica* (Umetsu et al. 1976). *C. japonica* hemolymph showed the presence of two different

agglutinins - anti-Bcj and anti-Xcj. N-acetyl derivatives strongly inhibited anti-Xcj while anti-Bcj was not inhibited by sugars (Umetsu et al. 1979).

Lectins specific for blood group B were found in three crabs: *C. acuta, C. bimaculatus,* and *C. japonica* (Umetsu et al. 1991). *Charybdis japonica* lectin specific for blood type B (CJA-B) and N-acetyl derivatives were identified in the hemolymph and was purified by affinity chromatography on Sephadex-200. The molecular weight of the lectin was about 300 kDa on gradient PAGE under non-denaturing condition, 19 and 38 kDa under reducing and non-reducing condition on SDS-PAGE. CJA-B gave a major band on a gradient PAGE (pH 3.5–10) with a pI of 5.0. The sugars palatinose and stachyose were the potent inhibitors. CJA-B specifically bound to the oligosaccharide terminal having α-glucose or α-galactose residues and the equatorial hydroxyl groups at the C-2 and C-3 positions of the sugar residues.

Humoral lectin was purified from the humoral fluids of *C. feriatus* using Sephadex G-100 column chromatography (Congjie et al. 2006). *C. feriatus* humoral lectin (CFL) was calcium dependent, sensitive to temperature and was inhibited by sugars D-mannose, D-fructose, D-arabinose and fucose. The molecular mass of CFL estimated by SDS-PAGE was found to be a single band of 40.8 kDa. Purification of CFL showed 45.70% recovery of total activity. The serum agglutinin of *Charybdis lucifera* was identified using mammalian erythrocytes (Meena et al. 2011a). The calcium dependent agglutinin gave highest hemagglutination activity with rabbit erythrocytes, bacteria *Vibrio fluvialis,* and trypsinized yeast.

6.4.4.2 Genus: *Scylla*

The agglutinin in the hemolymph of *Scylla serrata* was identified by Mercy and Ravindranath (1992). The hemolymph showed maximum affinity with human B, mouse, and rabbit erythrocytes. The optimum pH was between 7.0 and 7.5 and it was thermo labile with the maximum activity at 30 °C. The activity of *Scylla serrata* agglutinin was enhanced by Ca^{2+} but not by Mg^{2+}. The lectin was purified using bovine thyroglobulin agarose column and was characterized (Mercy and Ravindranath 1993). The purified lectin showed a molecular weight between 45 and 65 kDa on SDS–PAGE with a molecular weight of 55 kDa under non-reducing condition and a major and minor band of 30 kDa and 25 kDa, respectively, under reducing conditions. Sialidase treatment confirmed the NeuGc and α 2, 6 linkage specificity of lectin as revealed in erythrocyte binding affinity. Bovine and porcine thyroglobulin, BSM, bovine acid glycoprotein, and fetuin were the important inhibitors. De-O-acetylation of glycoprotein increased the inhibitory potency of *Scylla* lectin, which suggested the ability of the lectin to recognize the O-acetyl group. The immunological role of the lectin in *S. serrata* was analyzed by hemolysis and clearance assay (Mercy and Ravindranath 1994). The results revealed that the high agglutinating erythrocytes were cleared faster than the low agglutinating erythrocytes. Similarly the lectin-coated erythrocytes were cleared faster than the uncoated erythrocytes revealing the role of lectin in opsonization.

The *Scylla serrata* lectin "scyllin" was purified on D-GalNAc Separon column (Chattopadhyay and Chatterjee 1993). The activity of scyllin was optimum at pH 8.0 and was independent of divalent metal ions. It was inhibited by fetuin, human glycophorin, and ceruloplasmin. Low level of inhibition was observed with porcine stomach mucin, bird's nest glycoprotein, anti-thrombin III, and blood group O substances. The lectin purified by affinity chromatography was further purified by Mono-Q ion exchange column in FPLC using NaCl. Agglutination was noted in only one fraction out of the three fractions obtained.

The scyllin was further characterized (Chattopadhyay and Chatterjee 1997) and was found that it had a low molecular weight of about 4.8–5.0 kDa and a high content of acidic and neutral amino acids and mannose. The potent inhibitor of scyllin was ceruloplasmin and scyllin showed bactericidal activity. The hemolymph lectin of Thai marine crab, *Scylla serrata* was isolated by affinity chromatography and preparative electrophoresis (Kongtawelert 1998).

A natural C-type hemagglutinin specific to rabbit erythrocyte and non-reducing terminal glucose with 1–2 glucosidic linkage was identified in the serum of *Scylla serrata* (Jayaraj et al. 2010). The HA activity of the agglutinin was optimum at pH 7–9 and temperature 10–30. The agglutinin showed activity against bacteria and yeast cells.

A calcium dependent lectin "scyllin-2" was purified from *S. serrata* by successive 40% ammonium sulphate precipitation, affinity chromatography on asialo fetuin-Sepharose column and resource Q anion exchanger in FPLC system (Pramanik et al. 2010). Scyllin-2 was a homogenous lectin with a MW of 75 kDa as confirmed by ESI-MS-Q-TOF. Scyllin-2 exhibited mitogenic and antiproliferative effect.

Presence of multiple agglutinins was reported in the serum of the marine crab *Scylla serrata* (Philip et al. 2013). The hemagglutinins agglutinated buffalo, rat, human B, mouse, and rabbit erythrocytes and pathogenic bacteria. The optimum pH was found to be from 7 to 8.5. The HA activity was cation (Ca^{2+}, Sr^{2+}, and Mg^{2+}) dependent. GlcNAc, GalNAc, and ManNAc, mannan and laminarin were the carbohydrate inhibitors. The hemagglutinability was highly inhibited by the glycoprotein BSM.

Sialic acid specific lectin "Scyllin" from *Scylla serrata* was used to determine the amount of sialic acid in normal and cancer serum. It was found that the amount of sialoglycoproteins was higher in tumor tissues than the normal cells (Pinitglang et al. 2013).

A 24 kDa lectin named SSAP (a protein—*Scylla serrata* lectin) was purified from *Scylla serrata* (Krishnamoorthi et al. 2016). The isolated SSAP showed antibacterial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *B. subtilis*, *E. coli,* and *E. faecalis*.

The serum lectin of the crab *Scylla paramamosain* that agglutinated rabbit, dove, duck, and chicken erythrocytes, was inhibited by D-galactose, maltose, and rhamnose. The hemagglutination was optimum between pH 7 and 8 and temperature 30–40 °C (Qiang-Long et al. 2012).

Characterization of SpCTL-B, a C-type lectin present in the hemocytes of *Scylla paramamosain* revealed the presence of 1278 bp in SpCTL-B cDNA and 348 bp in

ORF and one CRD. Hepatopancreas showed the highest amount of mRNA transcripts of SpCTL-B. Exposure of *S. paramamosain* to LPS, *V. parahaemolyticus,* and WSSV enhanced the expression of mRNA in hepatopancreas and hemocytes. In the presence of calcium, SpCTL-B inhibited the growth of *S. aureus*, β-hemolytic *Streptococcus*, *E. coli*, *A. hydrophila,* and *V. alginolyticus*. D-mannose and LPS inhibited the agglutinability of rSpCTL-B. The role of SpCTL-B in the immunity of the crab was evident through its AMPs expression and phagocytosis (Wei et al. 2018).

SpGal (*Scylla paramamosain* galectin) bound rabbit RBCs with great avidity. A SpGal (galectin) gene from *Scylla paramamosain* was cloned. The SpGal cDNA of 3142 bp encoded a protein of 280 amino acids. The gill, hemocytes, and hepatopancreas showed high amount of mRNA transcripts of SpGal (Zhang et al. 2020). Challenging the animal with *V. alginolyticus* augmented the production of mRNA transcripts of SpGal, suggesting the role of this lectin in defense.

The SpCTL5, a C-type lectin with rabbit erythrocyte specificity was reported in the hepatopancreas of the crab *Scylla paramamosain* (Zhang et al. 2019b). The ORF of SpCTL5 is composed of 762 bp and has coded 253 amino acids. Hepatopancreas showed rich amount of SpCTL5 transcripts. In the presence of calcium, SpCTL5 recognized and agglutinated both Gram-negative and Gram-positive bacterial strains, revealing the antibacterial nature of this lectin.

A 16.7 kDa calcium dependent novel lectin, SpCTL6 with 144 amino acids, and pI value of 5.22 was detected in *S. paramamosain*. The cDNA of SpCTL6 contained 738 bp with an ORF of 486 bp and translated 161 amino acids. SpCTL6 was highly expressed in the zoea larva and during the moulting time of the crab. In the adult maximum level of the transcripts of SpCTL6 was observed in the testis. The binding capacity of SpCTL6 was observed with glycan, peptidoglycan, LPS, and lipoteichoic acid of bacterial cells. The rSpCTL6 increased hemocyte encapsulation and reduced bacterial endotoxin level (Qiu et al. 2021). Brachyuran crab *Scylla* has been extensively studied for the presence of lectins and is represented in Table 6.1.

6.4.4.3 Genus: *Portunus*

The serum of the estuarine crab *Portunus sanguinolentus* analyzed for the presence of agglutinin (Meena et al. 2011b) using mammalian erythrocytes, bacteria, and yeast as indicator cells, showed maximum agglutination activity with buffalo RBC, trypsinised yeast, *Vibrio fluvialis,* and *Vibrio alginolyticus*. The hemagglutination inhibition assay of the calcium dependent agglutinin revealed that the hemagglutinin was specific for non-reducing terminal glucose with α 1–2 glycosidic linkages.

The hemolymph lectin (Pp-Lec) with a MW of 155 KDa was purified from blue swimmer crab *Portunus pelagicus* (Jayanthi et al. 2017). Pp-Lec showed antibacterial activity against Gram-positive bacteria *B. pumulis*, *B. thuringiensis*, *E. faecalis* and Gram-negative bacteria *C. amalonaticus*, *V. parahaemolyticus*, *P. aeruginosa*, *P. vulgaris*, *C. murliniae*, *C. freundii*, *M. morganii.* The biofilm formation of Gram-negative bacteria was reduced with Pp-Lec at the concentration

Table 6.1 Investigation of lectin in the crabs of the Genus *Scylla*

Species	Agglutination RBC/bacteria/yeast	Inhibitors sugars/glycoproteins	Purification strategy	Molecular weight	Reference
S. serrata	Mouse > human B = rabbit	NeuGc, bovine and porcine thyroglobulin > BSM > bovine > acid glycoprotein > Fetuin	Bovine thyroglobulin Sepharose 4 B	30 kDa and 25 kDa	Mercy and Ravindranath, (1992, 1993, 1994)
	Human A, B and O, rabbit, rat, hamster, guinea pig, *B. cereus, E. coli*	Fetuin, human glycophorin Ceruplasmin	GalNAc-Separon	4.8–5.0 kDa	Chattopadhyay and Chatterjee (1993, 1997)
	–	BSM	Affinity chromatography preparative electrophoresis	–	Kongtawelert (1998)
	Rabbit, bacteria and yeast cells	1–2 glucosidic linkage	–	–	Jayaraj et al. (2010)
	–	Fetuin	40% ammonium sulphate precipitation and affinity chromatography on asialo fetuin-Sepharose column	75 kDa	Pramanik et al. (2010)
	Buffalo, rat, human B, mouse and rabbit, Gram-positive and Gram-negative bacteria	GlcNAc, GalNAc, and ManNAc, Mannan and Laminarin, BSM	–	–	Philip et al. (2013)
	Human O	BSM	BSM Sepharose 4B affinity chromatography column	100 kDa	Pinitglang et al. (2013)
	S. aureus, P. aeruginosa, K. pneumoniae, B. subtilis, E. coli, and E. faecalis	–	–	24 kDa	Krishnamoorthi et al. (2016)

S. paramamosain	Rabbit, dove, duck and chicken	D-galactose, maltose and Rhamnose	–	–	Qiang-Long et al. (2012)
	S. aureus, β-hemolytic *Streptococcus*, *E. coli*, *A. hydrophila* and *V. alginolyticus*	D-mannose and LPS	–	–	Wei et al. (2018)
	Rabbit	–	–	–	Zhang et al. (2020)
	Rabbit	–	–	–	Zhang et al. (2019b)
	V. alginolyticus	–	–	16.7 kDa	Wanlei Qiu et al. (2021)

of 50 μg/mL. The lectin Pp-Lec coated with silver nanoparticles (Pp-Lec-AgNp) appeared to be a 30–57 nm spherical molecule and exhibited higher antibacterial and antibiofilm properties than native Pp-Lec and silver nitrate. An antibiofilm property of Pp-Lec-AgNps was confirmed by exopolysaccharide (EPS) quantification index.

Portunus pelagicus lectin (PPL) of molecular weight of 27 kDa purified from the marine crab *Portunus pelagicus* could be a potential source of antibacterial drug (Chidhambaradhas et al. 2017) as it exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

A lectin, PtCTL1 was documented from hemolymph of *Portunus trituberculatus* (Lu et al. 2017). The CRD of this lectin contains four-conserved cysteine in the amino acid sequences. The cDNA of PtCTL1 was 702 bp long. The cDNA that encodes PtCTL1 was recombined with pET-21 a (+) with a C-terminal hexa-histidine tag fused in-frame and expressed in *E. coli* Origami (DE3). The mRNA transcripts of PtCTL1 were noted in hepatopancreas and hemocytes. PtCTL1 function as PRR and protects *Portunus trituberculatus* from bacterial infection.

Calcium dependent PtCTL-2 and PtCTL-3 lectins identified from *Portunus trituberculatus*, the swimming crab ORF encoded 485 and 241 amino acids, respectively. The motifs present in PtCTL-2 were EPR and QPD. The results of the microbial binding assay revealed the antimicrobial property of both rPtCTL-2 and rPtCTL-3 (Mengmeng et al. 2017).

A L-type lectin (PtLTL) with CRD containing four-conserved cysteine was recognized in swimming crab *Portunus trituberculatus* (Lu et al. 2018). The cDNA contained 1347 bp and the 714 bp cDNA fragment encoding PtLTL1 was combined with pET-21a (+) with a C-terminal hexa-histidine tag. The rPtLTL1 agglutinated both Gram-positive and Gram-negative bacteria. PtLTL1 mRNA transcripts were abundant in the hepatopancreas and hemocytes of the crab *Portunus trituberculatus*. The role of PtLTL as an opsonin was confirmed by challenging the crab with a bacterium, *Vibrio alginolyticus*.

A 654 bp cDNA of PtCTL4, a C-type lectin of *P. trituberculatus* with 480 bp ORF was found to possess one CRD with 2 calcium binding sites and 6 cysteine residues. The agglutination activity of PtCTL4 was maximum with rabbit erythrocyte. RT-PCR study showed high expression of PtCTL4 in hepatopancreas and low level in gonad, eyestalk, muscle, and hemocytes. Induction of *V. alginolyticus* into the crab caused a raise of PtCTL4 at 3 h, then gradual reduction at 12 h, and normal level of PtCTL4 at 48 h post-challenge. PtCTL4 showed calcium dependent bacterial agglutination with *B. aquimaris*, *S. aureus*, *M. lysodeik*, *V. alginolyticus*, *C. indologenes,* and *A. hydrophila* (Zhang et al. 2018).

The cDNA of the mannose-binding lectin of *P. trituberculatus* (PtMBL) contained 1208 bp with 732 bp ORF, coded 244 amino acids with QPD motif. Differential level of PtMBL transcripts were noticed in gill, eyestalk, and hemocytes of male and female crabs and on exposing the animal with bacteria *P. pastoris*, *V. alginolyticus,* and *M. luteus*. The antimicrobial potency of PtMBL was witnessed due to its agglutinability with yeast and bacteria. D-mannose and D-galactose inhibited the agglutination activity of PtMBL (Zhang et al. 2019a).

Table 6.2 Molecular characterization of *Portunus trituberculatus* lectin

Sl. no.	Name of the lectin	Number of base pairs in cDNA		Number of amino acids encoded	Motifs	Reference
		Full length	Open Reading Frame (ORF)			
1	PtCTL1	702	501	166	–	Lu et al. (2017)
2	PtCTL-2	–	–	485	EPR & QPD	Mengmeng et al. (2017)
3	PtCTL-3	–	–	241	EPR & QPD	
4	PtLTL	1347	774	257	–	Lu et al. (2018)
	PtLTL1		714	–	–	
5	PtCTL4	654	480	–	–	Zhang et al. (2018)
6	PtMBL	1208	732	244	QPD	Zhang et al. (2019a)
7	PtCLec1	873	–	176	YPD	Yue et al. (2020)
8	PtCLec2	–	–	–	QPD	Liu et al. (2021)

The cDNA of PtCLec1, a novel C-type lectin detected in the crab *P. trituberculatus* expressed 873 bp PtCLec1 coding 176 amino acids contained one signal peptide, one CRD with YPD motif. The expression of PtCLec1 transcripts was abundant in hepatopancreas. The rPtCLec1 recognized various PAMPs like LPS, peptidoglycan, glucan and *S. aureus*, *V. parahaemolyticus*, *V. alginolyticus*, *P. aeruginosa*, *P. pastoris*, *M. luteus* bacterial species. It showed strong agglutination activity towards yeast. The role of PtCLec1 in pathogen elimination and phagocytosis was also documented through in vivo and in vitro studies (Yue et al. 2020).

A C-type lectin (PtCLec2) showing AMP regulation and prophenoloxidase activation was highly expressed in *Portunus trituberculatus* intestine (Liu et al. 2021). PtCLec2 was galactose specific and had a single CRD with a QPD motif. The lectin agglutinated *V. alginolyticus*, *P. aeruginosa, S. aureus,* and *M. coccusluteus*, and rabbit erythrocytes in a Ca^{2+} dependent manner, and was suppressed by LPS, D-galactose, and D-mannose. Pathogen challenge increased the level of mRNA in the intestine. Knockdown of PtCLec2 decreased the transcription of two phagocytosis genes (PtArp and PtMyosin), three prophenoloxidase (proPO) system-related genes (PtPPAF, PtcSP1, and PtproPO), six antimicrobial peptides (AMPs) (PtALF4–7, PtCrustin1, and PtCrustin3), and PtRelish but upregulated the expression levels of PtJNK, PtPelle, and PtTLR. PtCLec2 showed antibacterial activity and mediated pathogen elimination through hemocyte phagocytosis, AMP synthesis, and proPO activation. The molecular characterization of *Portunus trituberculatus* lectin is depicted in Table 6.2.

6.4.5 Family: Polybiidae; Genus: Liocarcinus

A 700 kDa O-acetyl sialic acid specific lectin identified in the hemolymph of crab, *Liocarcinus depurator* was purified using a BSM Sepharose 4B gel chromatography

and eluted with 100 mM GlcNAc (Fragkiadakis and Stratakis 1997). The HA activity was highly inhibited by the sugar N-acetyl neuraminic acid with the interaction at C-5 group. The glycoprotein BSM that contains 9-O-Ac-and 8, 9-O-Ac-Neu5Ac was the good inhibitor. De-O-acetylation of the inhibitor BSM reduced the inhibitory potential of the glycoprotein with the lectin, revealing the O-acetyl sialic acid specificity. The lectin of *L. depurator* showed high affinity towards mouse erythrocytes.

The lectin agglutinated Gram-negative bacteria and inhibited the growth of *E. coli*. It also agglutinated and stimulated human lymphocytes. In vitro labeling of hemocytes and hepatopancreas with (^{35}S) methionine proved that the hepatopancreas was the site of synthesis of crab lectin. Hemolymph lectin was isolated from the hemolymph of two brachyuran crabs *Liocarcinus depurator* and *Potamon potamios* by adsorption on formalinized erythrocytes (Fragkiadakis and Stratakis 2000).

6.4.6 *Family: Xanthidae; Genus:* Atergatis

An agglutinin with specific affinity for dog erythrocytes was identified in *Atergatis reticulatus* and was characterized (Maghil and Mercy 1999). The lectin activity was heat labile and independent of cations (Ca^{2+} and Mg^{2+}). Hemagglutination inhibition assay recognized transferrin as the potent inhibitor.

A C-type lectin specific for rabbit erythrocytes with high activity at pH 7.5, temperature 35 °C and 2 mM calcium was reported in the serum of the marine crab *Atergatis subdentatus* (Denis et al. 2016).

A sialic acid specific CTL with two subunits of molecular masses 25 kDa and 50 kDa was purified from the hemolymph by biospecific adsorption from a marine crab *Atergatis ocyroe* (Elaya Bharathi et al. 2017).

A naturally occurring, calcium dependent, 216 kDa, O-acetyl sialic acid specific lectin (AiL) with antibacterial and anticancerous activity was purified from *Atergatis integerrimus* by using affinity chromatography and biospecific adsorption on formalinized erythrocytes. The lectin, AiL has three subunits: 70, 72, and 74 kDa. AiL agglutinated buffalo erythrocytes and its activity was reduced by inhibitors bovine submaxillary mucin, thyroglobulin, and raffinose (Elaya Bharathi et al. 2020).

6.4.7 *Family: Ocypoididae; Genus:* Ocypoda

A BSM specific hemolymph agglutinin capable of agglutinating chick, turkey, rabbit, rat, goat, dog, human A, B, O, cat, and cow erythrocytes was identified in the marine crab *Ocypoda platytarsis* (Mercy et al. 2004). The hemolymph agglutinated chicken erythrocytes with greater affinity. The agglutinin was calcium dependent and expressed maximum activity at pH 7.5 and temperature 35 °C.

6.4.8 *Family: Varunidae*

6.4.8.1 Genus*: Eriocheir*

The serum agglutinin from the crab *Eriocheir sinensis* was analyzed (Xue Lan et al. 2006). The serum agglutinin showed different agglutination capacity to erythrocytes of chicken, pigeon, rabbit, mouse, human A, B, and O, and binding capacity to bacteria such as *Micrococcus sarcina* and *Aeromonas hydrophila*. The serum agglutinin was calcium dependent, EDTA sensitive, and stable at pH from 5.0 to 9.0. Injection of bacteria (*M. sarcina*) into *E. sinensis* showed a peak value after 14 days that revealed the role of agglutinin as a nonspecific preventive factor.

The serum lectin that agglutinated four types of human erythrocytes and chicken erythrocytes was identified in the river crab *Eriocheir sinensis* and was characterized (Chun-Lua et al. 2008). The hemagglutinin was more sensitive to human A erythrocytes than other types of human erythrocytes. Maximum agglutination was observed at the temperature range of 4–37 °C and pH 5–8. Sugars such as D-glucose, D-fructose, sucrose, lactose, and maltose inhibited the hemagglutination activity at 1.0% concentration.

The cDNA of EsCTLDcp of the crab *Eriocheir sinensis* was 1258 bp long and the ORF was 975 bp long. This cDNA encoded a protein of 324 amino acids. The rEsCTLDcp agglutinated tested bacteria in the presence of calcium and cleared bacteria and the white spot syndrome virus (WSSV). The sequence of the cDNA of the lectins EsCTLDcp-1 and -2 of the crab *Eriocheir sinensis* were replicated by 5′ RACE. The expression of EsCTLDcp-2 was found in hepatopancreas, hemolymph, intestine, muscle, gill, gonad, and heart. Challenging the crabs with *A. hydrophila* induced maximum production of lectins at 12 h and tremendous reduction at 24 h post-challenge (Guo et al. 2011).

The cDNA of C-type *E. sinensis* Es-lectin with a MW of 11.8 kDa had 651 bp and the ORF 483 bp, which encoded 160 amino acids. Es-lectin showed the presence of three motifs: EPN, WND, FND. Higher expression of Es-lectin was noticed in the hemocytes than in stomach, intestine, gill, muscle, and hepatopancreas (Zhang et al. 2011).

EsLecF cDNA was cloned using expressed sequence tags (EST) of a hepatopancreatic cDNA and it comprised of a 477-bp ORF encoding a 158-amino-acid protein. LPS challenge augmented the expression of EsLecF transcripts in hepatopancreas of *E. sinensis.* The rEsLecF protein formed through prokaryotic expression showed binding activity towards various microorganisms, in the absence of calcium and induced the aggregation of microbial pathogens. Growth inhibitory assay and antibacterial assay were performed to assess the antibacterial effect of rEsLecF. The lectin inhibited the growth and also killed the bacteria. Also rEsLecF enhanced in vitro cellular encapsulation (Jin et al. 2013).

Two novel calcium dependent LTLs EsERGIC-53 and EsVIP36 were reported from *E. sinensis* (Huang et al. 2014a). The cDNA of EsERGIC-53 composed of 1955 bp and the ORF with 1506 bp that encodes a protein of 501 amino acids. The

cDNA of EsVIP36 composed of 3474 bp and the ORF comprising 984 bp, which encode a protein of 327 amino acids. The presence of ERGIC-53 and VIP36 in various tissues was confirmed by reverse transcription PCR. Infection with LPS (lipopolysaccharide), PGN (peptidoglycan), *S. aureus*, *V. parahaemolyticus,* and *A. hydrophila* increased the expression of mRNA transcripts of ERGIC-53 and VIP36 in hepatopancreas. Bacterium-binding experiment and sugar-binding assay confirmed the antibacterial property of the crab lectin and suggested its biological role as immune molecules.

EsCTL1 and EsCTL2, the CTLs of the crab *E. sinensis* were identified in the hepatopancreas by RT-PCR (Huang et al. 2014b). EsCTL1 and EsCTL2 possessed 169 and 164 amino acids respectively and one carbohydrate-recognition domain. Expression level of EsCTL1 and EsCTL2 was increased when challenged with LPS (lipopolysaccharide), PGN (peptidoglycan), *S. aureus,* and *A. hydrophila*. rEsCTL1 and rEsCTL2 agglutinated *S. aureus* (Gram-positive), *V. parahaemolyticus,* and *A. hydrophila* (Gram-negative) in the presence of calcium. In vivo studies revealed the fast clearance of *V. parahaemolyticus* from the circulation by rEsCTL1 and rEsCTL2, suggesting the antibacterial activity of these lectins.

A low-density lipoprotein receptor class A (LDLa) domain containing C-type lectin, EsCTLDcp was identified in the hepatopancreas of *E. sinensis* (Huang et al. 2014c).

The galectin, EsGal (*Eriocheir sinensis* galectin) was isolated from the Chinese mitten crab *Eriocheir sinensis*. Based on EST analysis, cDNA of EsGal was cloned using RACE technique. cDNA of EsGal was made up of 999 bp and the ORF coded a protein of 218 amino acids comprising a GLECT/Gal bind-lectin domain and a proline/ glycine rich low complexity region. The mRNA transcripts of EsGal were observed in hepatopancreas, gill, and hemocytes of the crab. Challenging the crabs with different microbes induced an increased mRNA expression. The recombinant EsGal (rEsGal) served as pathogen-associated molecular pattern (PAMPs) and bound with surface molecules like LPS (lipopolysaccharide), PGN (peptidoglycan), and GLU (glucan) of *E. coli*, *V. anguillarum*, *B. subtilis*, *M. luteus*, *S. aureus,* and *P. pastoris*. The agglutinating activity of the rEsGal with bacteria was inhibited by D-galactose and α-lactose. Thus, EsGal played vital role in the innate immune response by its capacity to recognisze and eliminate pathogens from the body of the crab (Wang et al. 2016).

6.4.8.2 Genus: *Varuna*

The hemagglutination activity of the hemolymph, hemocytes and extracts of hepatopancreas, muscle and gills was investigated in the crab *Varuna litterata* (Shoba and Rose 2016). Among the various tissues, hemolymph showed maximum HA activity with rat erythrocytes. The hemagglutination titer was influenced by the sex and size of the crabs. Maximum hemagglutinin activity was observed between pH 7.5 and 9.5 and temperature of 0–35 °C. The HA activity was unaffected by the addition of calcium, magnesium, and EDTA.

6.4.9 *Family: Leucosiidae; Genus:* Philyra

A NeuGc specific lectin PPA was identified in the hemolymph of the Korean marine crab, *Philyra pisum* (Kim et al. 2006) and was purified by gel filtration using Sephadex G-25 column and affinity column chromatography using BSM/ thyroglobulin coupled CNBr activated Sepharose 4B. The molecular weight of the isolated PPA lectin was 28.9 kDa. The agglutinability was inhibited by N-glycolyl neuraminic acid, bovine submaxillary mucin, and thyroglobulin. The PPA lectin showed antiproliferative effect against human hepatoma Hep3B, human bladder cancer, human stomach cancer SNU-Cl, human colon cancer SNU-1, and human lung cancer A549.

A lectin (PPL) with anticancerous activity on human lung cancer cells was detected in the hemolymph of the purse crab *Philyra pisum* (Na et al. 2011). The hemolymph lectin PPL agglutinated mouse, rat, and rabbit erythrocytes and the HA activity was stable from pH 4.0 to 8.0 and at temperatures below 40 °C. PPL was sensitive to chelating agent and lost its HA activity following dialysis with EDTA and was regained on addition of $CaCl_2$ or $MnCl_2$. MALDI-TOF mass spectrum analysis deduced the MW of PPL as 24.060 kDa. PPL was cation dependent with mitogenic activity on human lymphocytes and can function as lymphocyte or splenocytes stimulator.

6.4.10 *Family: Sesarmidae; Genus:* Episesarma

Naturally occurring lectins *Episesarma tetragonum* agglutinin 1 (ETA-1) and *Episesarma tetragonum* agglutinin 2 (ETA-2) were reported from the hemolymph of the mangrove crab *Episesarma tetragonum*. ETA-1 and ETA-2 were purified by using fetuin conjugated Sepharose 4B column and the lectin was eluted by elution buffer with N-acetyl glucosamine and EDTA, respectively. The 70 KDa ETA-1 showed the highest agglutination activity between pH 6.5 and 9.5 and temperature 0–40 °C. The calcium dependent ETA-1 was inhibited by glycoproteins fetuin, porcine submaxillary mucin, lactoferrin, and sugars N-acetyl glucosamine and N-acetyl mannosamine. The ETA-2 lectin showed highest agglutination activity between pH 8 and 9.5 and temperature 0–37 °C and was inhibited by BSM. De-O-acetylated BSM failed to inhibit the lectin ETA-2 suggesting it as O-acetyl sialic acid specific (Devi et al. 2013a).

Biological role of the lectin was studied by administering mammalian erythrocytes (models emulating foreign particles) of differential agglutinability. Administrating such foreign model induced and enhanced agglutinin production in the crab. Studies on the clearance of lectin-coated and uncoated erythrocytes from the hemocoel of the crab revealed that the lectin-coated erythrocytes were cleared faster from circulation than the uncoated ones indicating that the lectins may function as an

opsonin. Thus clearance of pathogens in crab may involve several specific processes, including "opsonization" of the foreign cells by the lectin (Devi et al. 2013b).

6.4.11 *Family: Cheiragonidae; Genus:* Erimacrus

The lectin EIL (*Erimacrus isenbekii* lectin) was purified by gel-filtration and anion-exchange chromatography from the crab *Erimacrus isenbeckii* (Na et al. 2007). The EIL lectin showed maximum activity with mouse and rabbit erythrocytes. The hemagglutinin activity was maximum at temperature 50 °C and pH from 5.6 to 8. The molecular weight of EIL was 116 kDa with two subunits of 54 and 62 kDa. EIL was inhibited by mannose and O-acetyl sialic acid specific bacterial pathogens.

6.4.12 *Family: Trichopeltariidae; Genus:* Trichopeltarion

A calcium dependent, N-acetyl neuraminic acid specific 96 kDa lectin was reported in the hemolymph of the marine crab *Trichopeltarion nobile* (Rama Devi et al. 2012). The lectin was purified by gel filtration chromatography using Sephadex G-75 column and the purified lectin showed maximum agglutination with rabbit erythrocytes at temperature ranging from 30 to 40 °C and pH 7–8.

6.4.13 *Family: Munidopsidae; Genus:* Shinkaia

The presence of multiple lectins in the serum of the deep sea vent endemic crab, *Shinkaia crosnieri* was identified (Fujiyoshi et al. 2015) by hemagglutination assay. The serum agglutinin activity was maximum with horse and rabbit erythrocytes and was inhibited by the sugars GlcNAc, Ara, Gal, GalNAc, Lac, and melibiose.

6.5 Conclusion

Host defense system is a primitive efficiency of all organisms. Since the invertebrates lack immunoglobulins, the humoral lectin a specific sugar-binding protein may be the functional analogue of an antibody and have been investigated in a number of species of crabs. Most of the crab lectins are calcium dependent C-type lectins and sialic acid specific siglecs. These sialic acid-specific lectins (siglecs) of the crab play an important biological role in recognizing and clearing invading pathogens. Sialic acid-specific lectins have gained much attention for biomedical application owing to their antimicrobial and anticancerous potential.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Chapter 7
Lectins in Penaeid Shrimps: Purification, Characterization, and Biological Significance

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Abstract Shrimps depend on cellular and humoral immune mechanism to eliminate pathogens present in its vicinity. Cell mediated response includes phagocytosis, melanization, encapsulation, opsonization, and cytotoxicity that will have a direct influence on the pathogens while humoral response includes humoral factors that are produced by the immune cells. In the absence of an adaptive immunity, they depend on the innate immunity to recognize and protect self from the non-self. The pathogens are recognized by the pathogen associated molecular patterns which activate the defense signaling pathway and trigger the expression of immune responsive genes. Among the pathogen recognition receptors, lectins—a humoral defense molecule recognizes and acts as an opsonin in phagocytic response. While the animal lectins are classified based on their structural characteristics as C-, I-, and P-type, Galectin, and Pentraxin, the shrimp lectins have been documented under seven groups as C-, I-, P-, and M-types, galectins, fibrinogen like domain lectins, and calnexin/calreticulin. These lectins have diverse structure, pattern of expression, and function in shrimp immunity. This chapter will lead into the identification, purification, characterization, and biological significance of shrimp and prawn lectins as reported by various researchers.

Keywords Cellular · Humoral · Immunity · Lectin · Shrimp · Pathogen recognition receptors

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Abbreviations

AMPs	Adenosine monophosphates
BSH	Brown shrimp hemagglutinin
BSM	Bovine submaxillary mucin
CD-MPR	Cation-dependent mannose-6-phosphate receptor
cDNA	Complementary deoxyribose nucleic acid
CI-MPR	Cation-independent mannose-6-phosphate receptor
CRD	Carbohydrate recognition domain
CRD1	Carbohydrate recognition domain 1
CRD2	Carbohydrate recognition domain 2
CTL	C-type lectin
CTLD	C-type lectin domain
CyHV-2	Cyprinid Herpesvirus 2
EDTA	Ethylene diamine tetra acetate
FBG domain	Fibrinogen like domain
FBN	Fibrinogen
FcCTL	*Fenneropenaeus chinensis* C-type lectin
Fc-hcL	*Fenneropenaeus chinensis* hepatopancreas lectin
Fc-hsL	*Fenneropenaeus chinensis* hepatopancreas specific lectin
FC-L	*Fenneropenaeus chinensis* lectin
FcLec1	*Fenneropenaeus chinensis* lectin 1
FcLec2	*Fenneropenaeus chinensis* lectin 2
FcLec3	*Fenneropenaeus chinensis* lectin 3
FcLec4	*Fenneropenaeus chinensis* lectin 4
FcLec5	*Fenneropenaeus chinensis* lectin 5
FcLec6	*Fenneropenaeus chinensis* lectin 6
Fclectin	*Fenneropenaeus chinensis* lectin
Fd	Fibrinogen-like domain
FIA	*Fenneropenaeus indicus* agglutinin
FLPs	Ficolin-like proteins
FML	*Fenneropenaeus merguiensis* lectin
FmLC3	*Fenneropenaeus merguiensis* C-type lectin 3
FMLC4	*Fenneropenaeus merguiensis* C-type lectin 4
FmLC5	*Fenneropenaeus merguiensis* C-type lectin 5
FmLC6	*Fenneropenaeus merguiensis* C-type lectin 6
FmLec	*Fenneropenaeus merguiensis* lectin
FmLFd	*Fenneropenaeus merguiensis* fibrinogen-like domain
FPLC	Fast protein liquid chromatography
FReD	Fibrinogen related domain
FREP	Fibrinogen related protein
FTIR	Fourier-transform infrared spectroscopy
GalNAc	N-Acetylgalactosamine
GH	Glycoside hydrolase

GluNAc	N-Acetylglucosamine
GTPase	Guanosine triphosphatase
HA	Hemagglutination Assay
HPLC	High performance liquid chromatography
IgG1	Immunoglobulin G sub-class 1
IHHNV	Infectious hypodermal and hematopoietic necrosis virus
L3	Lectin 3
LdlrCTL	Low-density lipoprotein receptor C-type lectin
Lectin-pCuS NPs	Lectin-pectin capped copper sulfide nanoparticles
LPS	Lipopolysaccharide
LsL	*Litopenaeus setiferus* lectin
Ls-Stylicin 1	*Litopenaeus stylirostris*-stylicin 1
LvCL1	*Litopenaeus vannamei* C-type lectin 1
LvCTL1	*Litopenaeus vannamei* C-type lectin 1
LvCTL3	*Litopenaeus vannamei* C-type lectin 3
LvCTL5	*Litopenaeus vannamei* C-type lectin 5
LvCTL-br 1	*Litopenaeus vannamei* C-type lectin-Brazil 1
LvCTL-br 2	*Litopenaeus vannamei* C-type lectin-Brazil 2
LvCTLD	*Litopenaeus vannamei* C-type lectin like domains
LvCTLU	C-type lectin gene in *Litopenaeus vannamei*
LvFrep	*Litopenaeus vannamei* fibrinogen related protein
LvGal	*Litopennaeus vannamei* galectin
LVL	*Litopenaeus vannamei* lectin
LvLec	*Litopenaeus vannamei* lectin
LvLectin-1	*Litopenaeus vannamei* lectin 1
LvLectin-2	*Litopenaeus vannamei* lectin 2
LvLT	*Litopenaeus vannamei* lectin transcriptome
LvLTLC1	*Litopenaeus vannamei* L-type lectin 1
LvPLP	*Litopenaeus vannamei* perlucin like protein
M6P	Mannose-6-phosphate
MaL	*Macrobrachium americanum* lectin
MALDI-TOF	Matrix-assisted laser desorption-ionization-time of flight
ManNAc	N-Acetyl mannosamine
MDA-MB-231	Epithelial human breast cancer cell line
Md-Lec	*Metapenaeus dobsoni* lectin
Mj	*Marsupenaeus japonicus*
MjCD-MPR	*Marsupenaeus japonicus* cation-dependent mannose-6-phosphate receptor
MjCTL	*Marsupenaeus japonicus* C-type lectin
MjFREP1	*Marsupenaeus japonicus* Fibrinogen related protein 1
MjFREP2	*Marsupenaeus japonicus* Fibrinogen related protein 2
MjGal	*Marsupenaeus japonicus* galectin
MjGCTL	*Marsupenaeus japonicus* gill C-type lectin
MjHeCL	*Marsupenaeus japonicus* hemocyte C-type lectin

MjLec	*Marsupenaeus japonicus* lectin
MjLTL1	*Marsupenaeus japonicus* L-type lectin 1
MmLec	*Metapenaeus monoceros* lectin
Mn-2TM-cLec	*Macrobrachium nipponense* 2 transmembrane C-type lectin
MnFico	*Macrobrachium nipponense* ficolin
MnLTL1	*Macrobrachium nipponense* L-type lectin 1
Mr(T)Lec2	*Macrobrachium rosenbergii* transcriptome lectin 2
Mr(T)Lec4	*Macrobrachium rosenbergii* transcriptome lectin 4
MrCTL	*Macrobrachium rosenbergii* C-type lectin
MrFico 1	*Macrobrachium rosenbergii* ficolin-like protein 1
MrFico 2	*Macrobrachium rosenbergii* ficolin-like protein 2
MrFico1	*Macrobrachium rosenbergii* Ficolin 1
MrFico2	*Macrobrachium rosenbergii* Ficolin 2
MrFREP	*Macrobrachium rosenbergii* fibrinogen related protein
MrL	*Macrobrachium rosenbergii* lectin
mRNA	Messenger ribonucleic acid
MrNV	*Macrobrachium rosenbergii* nodavirus
MrVIP 36	*Macrobrachium rosenbergii* vesicular integral protein of 36 kDa
NANA	N-Acetylneuraminic acid
Neu5Ac	N-Acetylneuraminic acid
NeuAC	N-Acetyl neuraminic acid
nMjHCTL	Endogenous *Marsupenaeus japonicus* hemocyte C-type lectin
ORFs	Open Reading Frames
PAMPs	Pathogen associated molecular patterns
PcL	*Procambarus clarkii* lectin
PcLT	*Procambarus clarkii* lectin transcriptome
PCR	Polymerase chain reaction
PjLec	*Penaeus japonicus* lectin
PjLec2	*Penaeus japonicus* lectin 2
PlFico1	*Pacifastacus leniusculus* ficolin-like protein 1
PlFico2	*Pacifastacus leniusculus* ficolin-like protein 2
PLP	Perlucin like protein
PmAV	*Penaeus monodon* antiviral protein
PmCLec	*Penaeus monodon* C-type lectin
PmFREP	*Penaeus monodon* fibrinogen-related protein
PmLec	*Penaeus monodon* lectin
PmLT	*Penaeus monodon* lectin transcriptome
PmMIP	*Penaeus monodon* melanization inhibition protein
PmTL5	*Penaeus monodon* tachylectin5
PRR	Pattern recognition receptor
Ps-Lec	*Penaeus semisulcatus* lectin
PSM	Porcine stomach mucin
PstCTL1	*Penaeus stylirostris* C-type lectin 1

qRT-PCR	Quantitative reverse transcription-polymerase chain reaction
RACE	Rapid amplification of cDNA ends
RBC	Red blood corpuscles
rCRD1	Recombinant carbohydrate recognition domain 1
rCRD2	Recombinant carbohydrate recognition domain 2
rFcCTL	Recombinant *Fenneropenaeus chinensis* C-type lectin
rFmLC5	Recombinant *Fenneropenaeus merguiensis* C-type lectin 5
rLvCTL1	Recombinant *Litopenaeus vannamei* C-type lectin 1
rLvCTL3	Recombinant *Litopenaeus vannamei* C-type lectin 3
rLvGal	Recombinant *Litopennaeus vannamei* galectin
rLvLec	Recombinant *Litopenaeus vannamei* lectin
rLvLTLC1	Recombinant *Litopenaeus vannamei* L-type lectin 1
rLvPLP	Recombinant *Litopenaeus vannamei* perlucin like protein
rMrCTL	Recombinant *Macrobrachium rosenbergii* C-type lectin
rPcLT	Recombinant *Procambarus clarkia* lectin transcriptome
rPcLT	Recombinant *Procambarus clarkii* Lectin Transcriptome
RT-PCR	Reverse transcription-polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate-Polyacrylamide gel electrophoresis
SP	Shrimp peptide
TCA	Trichloroacetic acid
TEM	Transmission electron microscope
UTR	Untranslated region
WSSV	White spot syndrome virus
YHV	Yellow head virus

7.1 Introduction

Shrimps are decapod crustaceans with elongated body, long antennae, and well developed pleopods. They are widespread and can be located in the sea floor, rivers, and lakes. Shrimps/prawns are classified under 33 genera with around 2500 species (Holthuis 1980). Penaeids come under five penaeidean families: Solenoceridae, Aristidae, Penaeidae, Sicyoniidae, and Sergestidae and three caridian families: Pandalidae, Crangonidae, and Palaemoninae.

Aquatic invertebrates like shrimps and prawns contain some degree of microbes in their hemolymph and the microbiota is controlled in healthy animals (Wang et al. 2014). Due to the nature of the environment, penaeid shrimps are susceptible to invaders like virus, bacteria, fungi, and parasite (Zhang et al. 2021a, b). Shrimps lack the adaptive immunity and they depend on physical barriers to fight against infectious microbes (Loker et al. 2004; Hanington et al. 2010; Amparyup et al. 2012). Lectins, antimicrobial proteins, and other humoral molecules play a vital part in detecting and eliminating any pathogenic influence (Christophides et al. 2004).

Shrimp defense mechanism relies on the intrinsic immunity that consists of PRR that can bind to the surface of pathogens. They activate the immune signaling pathways that inturn activates the cellular and humoral response (Huang et al. 2008; Li et al. 2019). The cellular immune response includes phagocytosis and apoptosis by hemocytes while the humoral response involves phenoloxidase system, lectins, and antimicrobial peptides in the hemolymph. The hemocytes in crustaceans play a key role in immunity by recognition, phagocytosis, melanization, and cytotoxicity (Jiravanichpaisal et al. 2006; Huang et al. 2020; Kulkarni et al. 2021). In majority of the crustaceans, hemocytes namely hyaline cells, semigranular cells, and granular cells in the hemolymph play a key part in destroying and clearing the invading pathogens (Bauchau 1981; Johansson et al. 2000). The important role played by hepatopancreas and hemocytes in crustacean immunity was reported by Jiravanichpaisal et al. (2006).

7.2 Crustacean Lectins

Crustaceans are protostomian invertebrates which inhabit both aquatic and terrestrial environment and are subjected to opportunistic pathogens, disease, and other stress factors. The crustacean defense system depends on the innate immune response to recognize the pathogens and participate in the identification of non-self. When the innate immune system is activated, both cellular and non-cellular immune components like hemocytes, clotting factors, diverse classes of pathogen recognition receptors (PRRs), lectins, antimicrobial peptides, and effector molecules activate distinct pathways to destroy the invading pathogens (Marques and Barracco 2000; Vasta et al. 2007; Vazquez et al. 2009). The defense processes includes agglutination, phagocytosis, encapsulation, activation of oxidative burst, production of reactive oxygen species, opsonization, and clearance of pathogens (Sanchez et al. 2014; Sierra et al. 2005; Luo et al. 2006; Sivakamavalli and Vaseeharan 2014; Alpuche et al. 2009; Zhang et al. 2011).

Among various immune components, agglutinins or lectins that can recognize specific carbohydrate structures are documented from crustaceans like barnacles, cray fish, shrimps, prawns, crabs, and lobsters (Hall and Rowlands 1974; Kopacek et al. 1993; Luo et al. 2006; Mercy and Ravindranath 1993; Alpuche et al. 2005; Battison and Summerfield 2009; Devi et al. 2013; Elaya Bharathi et al. 2020; Bai and Rose 2020). Lectins are produced by different tissues like hepatopancreas, hemocytes, gills, ovary, muscles, brain, heart, and stomach (Vazquez et al. 1997; Liu et al. 2007; Kong et al. 2008). Of the different tissues, hemocytes and hepatopancreas were identified as primary sites involved in the synthesis of lectins (Jiravanichpaisal et al. 2006; Gross et al. 2001; Soderhall 2010). Immunochallenge with different strains of bacteria, virus, or other stress factors have resulted in the production of induced lectins and the gene expression of lectins have been observed in organs and tissues like hepatopancreas, muscle, eyestalk, and cuticle (Ma et al. 2007; Gross et al. 2001; Leu et al. 2007).

Lectins identified in crustaceans have diverse immunological functions within their innate immune system. Agglutination of pathogens by recognizing specific carbohydrate domains expressed on the cell surface is a significant activity noted among crustacean lectins (Vasta et al. 2012). Although several crustacean lectins can recognize a broad range of carbohydrate moieties, many have high specificity for N-acetylated sugars (sialic acid specific—NeuAc and NeuGc) (Hall and Rowlands 1974; Mercy and Ravindranath 1993; Fragkiadakis and Stratakis 1997; Denis et al. 2003), ManNAc, GlcNAc, GalNAc, and simple sugars such as galactose and mannose residues (Alpuche et al. 2005). Lectins can recognize carbohydrate motifs expressed on the cell surface thus aiding in the recognition and elimination of many crustacean pathogens (Vazquez et al. 1994). Shrimp lectins like LvCTL1 (Zhao et al. 2009), LvLectin – 2 (Wei et al. 2012) exhibited antiviral activity against white spot syndrome virus and antimicrobial activity against Gram +ve bacteria, Gram −ve bacteria, and fungi. Lectins from *Procambarus clarkii* activated the proPO system enhancing phagocytosis (Chen et al. 2013) and hemocyte encapsulation. Many hemolymph lectins from decapods could also act as PRRs (Marques and Barracco 2000). Thus, lectins are found to participate and contribute to the crustacean's innate immune defense against pathogen challenge.

Crustacean lectins demonstrate antibacterial, antiviral, antifungal, and antiproliferative properties owing to their inherent potency to recognize the glycoconjugates expressed on the cell surface and has gained immense biomedical potential. Anticancerous property was observed in a fucose specific lectin isolated from shrimp *Fenneropenaeus indicus* against MCF-7 cells (Chatterjee et al. 2017), Scyllin-2 from crab *Scylla serrata* against HepG2 cells (Pramanik et al. 2010), PPA lectin from crab *Philyra pisum* against human cancer cell lines Hep3B (hepatoma), SNU-Cl (stomach cancer), SNU-1 (colon cancer), and A549 (lung cancer) (Kim et al. 2006) and an N-acetylated sugar specific lectin—INoL from slipper lobster *Ibacus novemdentatus* against breast cancer cells (MCF-7 and T47D), ovarian cancer (HeLa), and colonic cancer ($CaCo_2$) (Fujii et al. 2017). Antimicrobial activity was observed in shrimp *Litopeneus vannamei* (Tian et al. 2018), *Fenneropenaeus chinensis* (Lai et al. 2013), *Penaeus semisulcatus* (Sivakamavalli and Vaseeharan 2014), crab *Portunus pelagicus* (Chidhambaradhas et al. 2017), *Scylla serrata* (Chattopadhyay and Chatterjee 1993; Krishnamoorthi et al. 2016), and lobster *Jasus novaehollandiae* (Kamiya et al. 1994). However, there is far more to understand on how these crustacean lectins with specific carbohydrate recognition could regulate their immune response, which can open up pathways to cure or prevent diseases.

7.3 Lectins as Defense Molecules in Shrimps

Lectins are glycoproteins ubiquitous in nature with specific carbohydrate binding property (Sharon and Lis 1972). Lectins bind to the carbohydrate moieties on the surface of microbes, erythrocytes, mammalian cells, and other cells of invertebrates

causing agglutination and mediate in recognition of foreign invaders and biological processes including protein trafficking, cell signaling, and complement activation (Ogawa et al. 2011; Jyotirmaya et al. 2020; Wang et al. 2020). From the data available in GenBank shrimp transcriptome data, at least six types of lectins were reported in shrimps that include C-, L-, M-, P-type lectins, galectins, and fibrinogen-like domain lectins. The diversity in lectin types is provided by the conserved sequence motifs displayed in their carbohydrate recognition domain (CRD). These lectins are also included under PRR due to their capability to bind to different carbohydrate moieties on the pathogen surface.

7.3.1 C-Type Lectins (CTLs)

CTLs designated so to denote their calcium dependency play an important part in the innate immune response of shrimps by promotion of phagocytosis, nodule formation, melanization, encapsulation, and prophenoloxidase activation (Luo et al. 2006). Number of CTLs has been reported in several members of Penaeids involving in innate immunity. C-type lectins were identified in *F. chinensis*, *L. vannamei*, *P. monodon,* and *M. japonicus* (Sun et al. 2008; Zhang et al. 2009a; Wang et al. 2009a; Xu et al. 2010). These lectin families differ in the composition of carbohydrate recognition domain (CRD) that act as pattern recognition receptors (PRRs). Many shrimps have one or many CRDs with other additional modules (Wang and Wang 2013). Shrimp lectins like, *Lv*Lectin – 1 and *Lv*Lectin – 2 from *Litopenaeus vannamei* (Wei et al. 2012) generally have a signal peptide (SP), carbohydrate recognition domain (CRD) with GPN (Gln122.Pro123.Asn124) or GPD (Gln128. Pro129.Asp130) aminoacid motifs. These motifs are expected to have specificity towards carbohydrates like mannose / galactose and also calcium. *Lv*CTL 4.2 from *L. vannamei* (Huang et al. 2021) has a mutated mannose binding region EPA (Glu – Pro – Ala) in its CRD domain (Fig. 7.1A). Lectin like *Mj*lectin from *Marsupenaeus japonicus* (Zhang et al. 2015) has two dissimilar CRDs of unequal length, namely, CRD1 and CRD2. CRD1 has the LPN motif (Leu134. Pro135. Asn136) and CRD2 with EPN motif (Glu299.Pro300.Asn301), *Fc* – Lec 2 of *Fenneropenaeus chinensis* (Zhang et al. 2009a) possess CRD1 with GPD (Gln134.Pro135.Asp136) and EPN motif (Glu298.Pro299.Asn300) (Fig. 7.1B). Structures of CRDs are usually stabilized by two conserved disulfide bridges. Diverse role of these lectins in shrimp – pathogen interaction depends on CRD's ability to recognize the carbohydrate moieties. Shrimp C-type lectins have been mostly isolated from the hemolymph, hepatopancreas, and hemocytes and widely distributed in multiple tissues (Zhao et al. 2009).

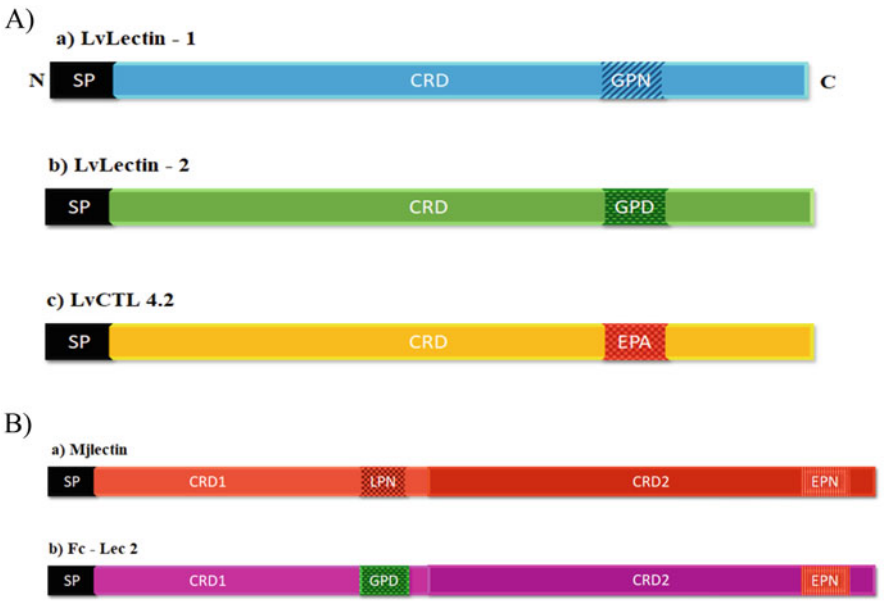

Fig. 7.1 Simplified representation of structural diversity of C-type lectins in Shrimps. (**A**) Single CRD C-type lectins. Examples: (a) LvLectin—1 and (b) LvLectin—2 of *Litopenaeus vannamei* (Wei et al. 2012). These lectins generally have a signal peptide (SP), carbohydrate recognition domain (CRD) with GPN (Gln_{122}.Pro_{123}.Asn_{124}) or GPD (Gln_{128}.Pro_{129}.Asp_{130}) amino acid motifs. These motifs are expected to have specificity towards carbohydrates like mannose / galactose and also calcium. (c) Another C-type lectin, LvCTL 4.2 from *L. vannamei* (Huang et al. 2021) is found to have a mutated mannose binding region EPA (Glu–Pro–Ala) in its CRD domain. (**B**) Dual CRD C-type lectins. Example: (a) Mjlectin of *Marsupenaeus japonicus* (Zhang et al. 2015). This lectin has two dissimilar CRDs of unequal length, namely, CRD1 and CRD2. CRD1 has the LPN motif (Leu_{134}. Pro_{135}. Asn_{136}) and CRD2 with EPN motif (Glu_{299}.Pro_{300}.Asn_{301}) (b) Fc – Lec 2 of *Fenneropenaeus chinensis* (Zhang et al. 2009a). CRD1 with GPD (Gln_{134}.Pro_{135}.Asp_{136}) and EPN motif (Glu_{298}.Pro_{299}.Asn_{300}). Structures of CRDs are usually stabilized by two conserved disulfide bridges

7.3.2 L-Type Lectins

The L-type lectins have luminal CRD with leguminous lectin like domain (JQ804933) with high specificity to saccharides. Identified L-type lectins namely *Mj*LTL1 and *Mj*LTL2 in *Marsupenaeus japonicus* (Xu et al. 2014, 2020) *Mn*LTL1 in *Macrobrachium nipponense* (Xiu et al. 2015), *Lv*LTLC1 in *Litopenaeus vannamei* (Tian et al. 2018) *Mr*VIP 36 in *Macrobrachium rosenbergii* (Huang et al. 2018), and *Pc*L—lectin in *Procambarus clakii* (Dai et al. 2016) (Fig. 7.2) expressed ubiquitously in almost all tissues involving in antibacterial immune response.

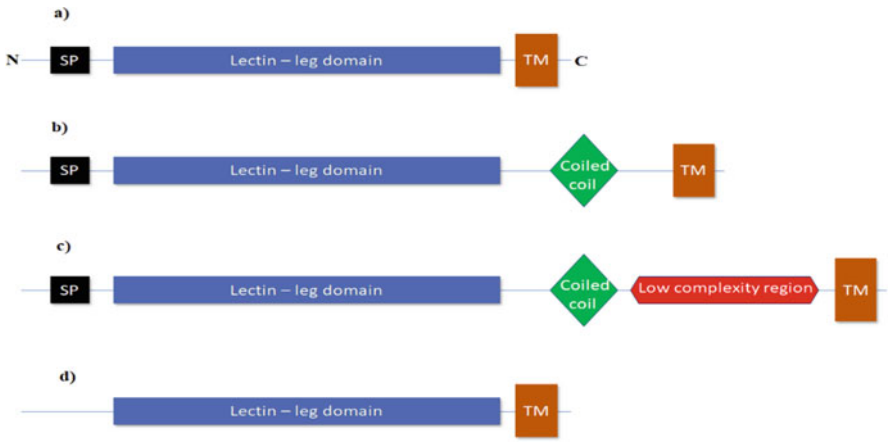

Fig. 7.2 Representative domain structure of L-type lectins in Penaeids. (**a**) L-type lectins, namely *Mj*LTL 1 (*M. japonicus*, Xu et al. 2014), *Lv*LTLC1 (*L. vannamei*, Tian et al. 2018), *Mn*LTL 1 (*M. nipponense*, Xiu et al. 2015) have a signal peptide region (SP), luminal CRD with the leguminous lectin like domain (LTLD) and a transmembrane domain at its carboxyl end. (**b**) *Mj*LTL 2 (*M. japonicus*, Xu et al. 2020) has an additional coiled coil structure. (**c**) *Pc*L—lectin (*Procambarus clarkii*, Dai et al. 2016) with low complexity region and (**d**) *Mr*VIP 36 (*M. rosenbergii* vesicular integral membrane protein 36, Huang et al. 2018)

7.3.3 M-Type

The M-type lectins contain glycoside hydrolase family 47 (GH47) domain (JQ804932) (Fig. 7.3A). M-type lectins are less identified in shrimps and so far, reported in *M. nipponense* (Xiu et al. 2015) and *M. japonicus* (Zheng et al. 2020). These show abundant expression in intestine, moderate in gills and hepatopancreas, and lower in other tissues (Xiu et al. 2015).

7.3.4 P Type

P-type lectin comprises cation-dependent and cation-independent mannose 6-phosphate receptor (CD-MPR and CI-MPR) that can recognize as well as bind specifically to mannose 6-phosphate (M6P). These are multifunctional type I transmembrane glycoproteins with three major domains, namely, signal peptide, M6P receptor domain/ extracytoplasmic region, and a transmembrane domain (Fig. 7.3B). *Mj*CD-MPR was identified in *Marsupenaeus japonicus*. Its extracytoplasmic region bound to several polysaccharides like lipoteichoic acid, lipopolysaccharides, and peptidoglycan and thus could function as PRR in the shrimp for bacterial recognition (Zhang et al. 2018).

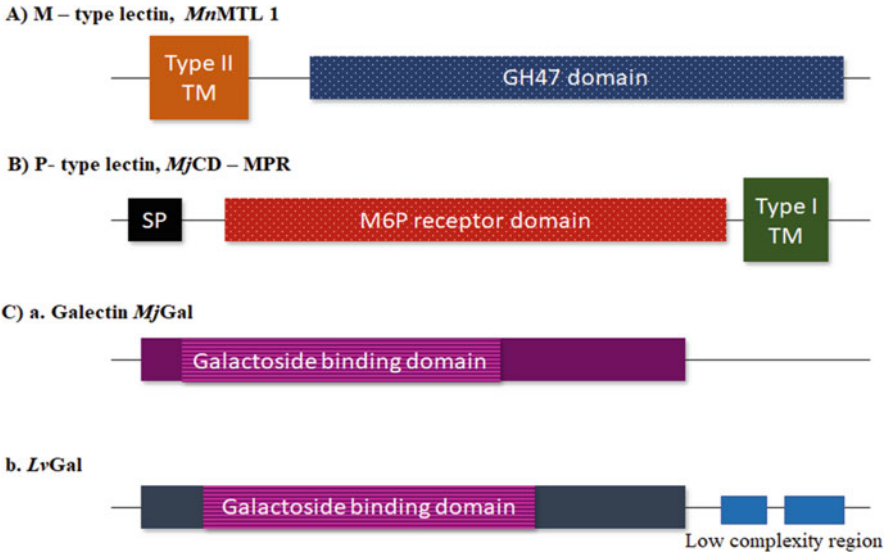

Fig. 7.3 (**A**) M-type lectin *Mn*MTL 1 (*M. nipponense*, Xiu et al. 2015) with type II transmembrane region at N-terminal end, GH 47 domain (glycoside hydrolase family 47) at the C-terminal end. (**B**) P-type lectin, *Mj*CD – MPR (*Marsupenaeus japonicus*, Zhang et al. 2018) with a SP domain, M6P receptor domain, and type I TM domain at C-terminal end. (**C**) (**a**) Galectin *Mj*Gal (*Marsupenaeus japonicus*, Shi et al. 2014) with CRD having the galactoside binding domain in the N-terminal half of the polypeptide chain. (**b**) *Lv*Gal (*Litopenaeus vannamei*, Hou et al. 2015) with its galactoside binding domain and long low complexity regions

7.3.5 Galectins

Galectins (β-galactoside binding lectins) was designated formerly as S-type lectins to indicate the sulfhydryl dependency for their functional activity. Galectin, *Mj*Gal identified from *Marsupenaeus japonicus* could function both in pathogen recognition and as an opsonin promoting bacterial clearance (Shi et al. 2014). *Lv*Gal from *Litopenaeus vannamei* also served as a potential PRR and their several amino acid residues were found to involve in dimerization (Hou et al. 2015). Galactoside binding domain of both the galectins are at their N-terminus, *Mj*Gal (10–143), and *Lv*Gal (16–142) and have no signal peptides and transmembrane domain. *Lv*Gal has a long low complexity region (Fig. 7.3Ca, b).

7.3.6 Lectins with Fibrinogen Like/Related Domain

Lectins with fibrinogen (FBN) like/related domain (FReDs) with complex architectures have been identified in few penaeids. Lectins with fibrinogen like/related domain are also known as ficolins / FREPs. Ficolins have, collagen-like domain

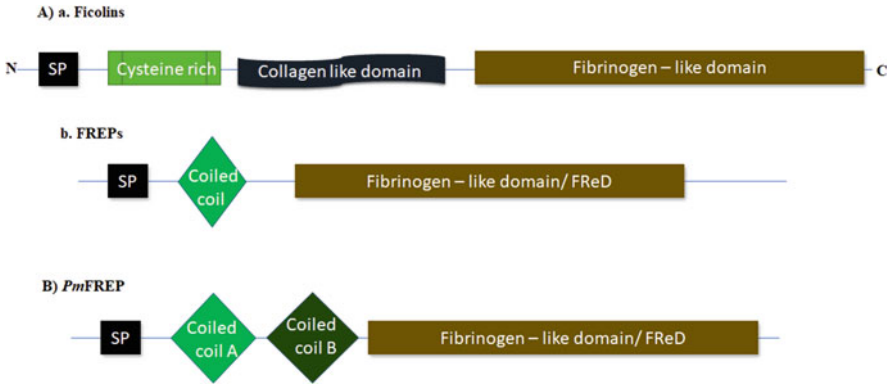

Fig. 7.4 (**A**) Ficolins vs. FREPs: (**a**) Ficolins are characterized by collagen like domain whereas (**b**) other lectins with fibrinogen related domain have no collagen like domain. (**B**) Recombinant *Pm*FREP (*P. monodon*), an example of lectins with fibrinogen related domain

that makes ficolins trimerize, cysteine rich N-terminus that oligomerize trimerized ficolins by disulfide linkages and the FBN-like domain at the C-terminus that commonly binds to N-acetyl sugars. FREPs have a coiled region and a FReD domain. Absence of collagen-like domain differentiates FREPs from the ficolins (Fig. 7.4A). Shrimps are found to have ficolin-like proteins (FLPs) and FREPs. Two FLPs from *Pacifastacus leniusculus* (Wu et al. 2011), *Mj*FREP 1 and 2 identified in *M. japonicus* (Chai et al. 2012), *Mr*Fico 1and 2, *Mr*FREP in *M. rosenbergii* (Zhang et al. 2014; Han et al. 2018), *Fm*LFd in *F. merguiensis* (Senghoi et al. 2019), *Mn*Fico 1, 2, and 3 in *Macrobrachium nipponense* (Li et al. 2017; Qin et al. 2020), *Pm*FREP in *P. monodon* (Singrang et al. 2021) have no collagen like domains and had varied domains in N-terminal region (Fig. 7.4B). All these proteins functioned as potential PRRs in the identified penaeid's immune system.

7.4 Distribution of Lectins in Tissues of Shrimps

Shrimps that inhabit the aquatic environment are highly challenged with pathogens and hazardous material which makes them susceptible to infectious diseases and stressors. Nature has provided an efficient immune mechanism and lectins which are humoral defense molecules are synthesized by various tissues to combat the stressful situation. On challenge with microbes, the lectins are induced and they help in the clearance of the pathogens by phagocytosis, opsonization, melanization, and production of humoral factors (Sritunyalucksana and Soderhall 2000) to protect them against invading pathogens.

7.4.1 Hepatopancreas/Stomach/Intestine

These tissues are a source of many C-type lectins and the expression of the genes was highly observed in the hepatopancreas. Lectins that are expressed in the hepatopancreas has been identified as FcLec 1, 2, 3, 4, 5, and 6 in *F. chinensis* (Sun et al. 2008; Zhao et al. 2009; Wang et al. 2009a, b; Xu et al. 2010), LvLT, LvLec, LvCTL1 in *L. vannamei* (Zhao et al. 2009; Zhang et al. 2009b), PMAV, PmLT, PmLec in *P. monodon* (Luo et al. 2007; Ma et al. 2008; Soonthornchai et al. 2010), FmLC in *F. merguiensis* (Rattanaporn and Utarabhand 2011), and PsTCTL1in *Penaeus stylirostris* (Dhar et al. 2003).

7.4.2 Hemocytes

Hemocytes that circulate in the hemolymph constantly produce lectins when exposed to microbes. The lectins produced by hemocytes are Fc lectin in *F. chinensis* (Liu et al. 2007), LvLec in *L. vannamei* (Zhang et al. 2009b), PmLec in *P. monodon* (Soonthornchai et al. 2010), and MjLec A and B in *M. japonicus* (Song et al. 2010).

7.4.3 Gills

Lectins FcLec4 from *F. chinesis* (Wang et al. 2009b) LvCTl and LvCTLD of *L. vannamei* (Costa et al. 2011; Junkunlo et al. 2011) have been isolated from the gills of shrimps.

Heart, ovary, lymphoid organs, and muscle have also been identified as a source of lectins as reported in *L. vannamei, P. clarkii*, *M. nipponense, F. chinensis,* and *Marsupenaeus japonicus* (Zhang et al. 2009b; Junkunlo et al. 2011; Wang and Wang 2013; Wang et al. 2014; Sun et al. 2014; Hou et al. 2015; Xiu et al. 2015, 2016; Xu et al. 2020).

7.5 Identification of Lectins

Penaeid shrimps belong to class: crustacea, order: Decapoda and suborder: Macrura. Economically important species of shrimps and prawns belong to this suborder and have been extensively studied for the presence of lectins (Table 7.1) and the gene expression of the lectins along with its functional significance is elucidated (Table 7.2).

Table 7.1 Identification, characterization, and biological significance of shrimp lectins

Name of shrimp/ lectin identified	Agglutination and binding specificity to erythrocytes/ glycoproteins/ sugars/microbes	Purification strategy and molecular weight of the lectin	Biological significance	Reference
Cray fish				
Procambarus clarkii	Rabbit erythrocytes Marine bacteria	150 kDa	–	Miller et al. (1972)
Pacifastacus leniusculus	Trypsinized rabbit erythrocytes PSM, BSM, fetuin and LPS from *E. coli* K 235	420 kDa	–	Kopacek et al. (1993)
Procambarus clarkii C-type lectin, PcLT	*Vibrio alginolyticus* and WSSV	–	Increase in mRNA and immunological factors, peroxinectin, phenoloxidase and superoxide dismutase after challenge with *Vibrio alginolyticus* and WSSV	Chen et al. (2013)
Penaeus monodon				
Sialic acid specific lectin -Monodin	*Vibrio vulnificus*	Fetuin agarose column-420 kDa FPLC anion exchange chromatography-27 kDa	–	Ratnapo and Chulavatnatol (1990, 1992)
Vibrio alginolyticus induced lectin	Sheep erythrocytes	–	–	Adams (1991)
C-type lectin PmLec	Bacterial LPS Gram negative bacteria	–	–	Luo et al. (2006)
Penaeus japonicus				
Calcium dependent lectin Calcium independent lectin	Calf, horse, swine, sheep and rabbit erythrocyte D-idose and NANA	–	–	Kondo et al. (1992)

	Sheep and rabbit erythrocyte D-idose and D-mannosamine			
Multiple lectins	Horse, sheep, human erythrocytes	PSM—Sepharose 6- B column followed by FPLC on a mono Q column		Muramoto et al. (1995)
L3	Rat and mice erythrocytes, galactose, mannose, arabinose, glucosamine and GluNAc *Vibrio alginolyticus* HW283	–	–	Shanon et al. (2002)
PjLec	Mammalian erythrocytes Gram-positive and gram-negative bacteria ManNAc, Neu5Ac, lipopolysaccharides and bovine submaxillary mucin	Affinity chromatography with fetuin-Sepharose 37 kDa	–	Yang et al. (2007)
Mannose-binding C-type lectin (PjLec2) from	Gram-negative bacteria *Vibrio alginolyticus* and *Aeromonas hydrophila*	–	EPN (Glu-pro-Asn) motif for mannose recognition	Gao et al. (2020)
Marsupenaeus japonicus				
L-type lectin 1 -MjLTL1	Gram-positive and gram-negative bacteria	–	Clearance of *V. anguillarum* –functions as an opsonin	Xu et al. (2014)
MjGCTL (nMjHCTL) Gills	–	–	Bacterial phagocytosis by hemocytes	Alenton et al. (2019)
L-type lectin—MjLTL2	Gram-positive and gram-negative bacteria	–	Upregulation of MjLTL2 upon injection of *V. anguillarum* and WSSV	Xu et al. (2020)
MjHeCL	–	–	Inhibits bacterial proliferation	Wang et al. (2014)

(continued)

Table 7.1 (continued)

Name of shrimp/ lectin identified	Agglutination and binding specificity to erythrocytes/ glycoproteins/ sugars/microbes	Purification strategy and molecular weight of the lectin	Biological significance	Reference
C-type lectin—CTL33	–	–	CTL33 promotes intestinal bacteria and regulates the homeostasis of the microbiota	Zhang et al. (2021a, b)
P. californiensis				
Fraction A	Mouse erythrocytes	GalNAc agarose column	–	Vargas-Albores et al. (1992)
BSH-1 (fraction B)	Human and mouse erythrocytes	Sepharose 6B column 180 kDa	–	
P. longirostris	NeuAc and galactose GalNAc *Pseudomonas aeruginosa*	Adsorption on formalinized rabbit erythrocytes 27 kDa and 36 kDa	–	Fragkiadakis and Stratakis (1995)
P. indicus	Human A erythrocyte Acetylated aminosugars	–	–	Maheswari et al. (1997)
	–	Gel filtration chromatography 181 kDa	–	Jayasree (2001)
FIA-*Fenneropenaeus* (*Penaeus*) *indicus* lectin	LPS obtained from gram negative bacteria	Affinity chromatography on GluNAc Sepharose 6B column 200 kDa	–	Maheswari et al. (2002)
Penaeus semisulcatus *Ps*-Lec	Mammalian erythrocytes Gram-positive and gram-negative bacteria Fungi- *Candida albicans*	Affinity column chromatography with mannose coupled Sepharose CL-4B column 37 and 118 kDa		Sivakamavalli and Vaseeharan (2014)
Semisulcatus-lectin	Human erythrocytes	Affinity column chromatography with mannose coupled Sepharose CL-4B column	Antibacterial activity against *Vibrio parahaemolyticus* and *Aeromonas hydrophila*	Preetham et al. (2020)

Litopenaeus setiferus LsL	Guinea pig and rabbit erythrocytes Sialylated O- glycosylated proteins and N- acetylated sugars	Affinity chromatography on gluteraldehyde fixed stroma from rabbit erythrocytes 291 kDa	–	Alpuche et al. (2005)
***Litopenaeus schmitti*—**sialic acid specific lectin	Sialic acid (Neu5Ac) and O-sialoglycoconjugates (BSM)	Affinity chromatography on fetuin—agarose column 220 kDa band with two subunits of 31 and 34 kDa	–	Cominetti et al. (2002)
Litopenaeus vannamei				
C-type lectin	Rabbit erythrocytes Galactose, mannose, fructose and glucose	–	–	Leung et al. (2005)
LVL	Human A, B, O, mouse, and chicken erythrocytes GalNAc, GluNAc, and NeuAc, glycoproteins bovine submaxillary mucin and Fetuin Gram-negative bacteria	Affinity chromatography using fetuin-coupled agarose 172 kDa consisting of 32 and 38 kDa subunits	–	Sun et al. (2007)
C-type lectin, *LvCTL1*	Mannose	–	Anti-white spot syndrome virus (WSSV) activity	Zhao et al. (2009)
C-type lectin—LvCTL3	*V. alginolyticus, V. parahaemolyticus, B. subtilis*	–		Li et al. (2014)
LdlrCTL	Bacteria and fungi	–	Antibacterial and antifungal response	Liang et al. (2019)
Fenneropenaeus merguinensis				
FML	Rabbit and rat erythrocytes NeuAc	Fetuin agarose column 316.2 kDa	–	Rittidach et al. (2007)
FML	Porcine stomach mucin and fetuin *Vibrio harveyi*, *Vibrio parahemolyticus,* and *Vibrio vulnificus*	–	–	Utarabhand et al. (2007)

(continued)

Table 7.1 (continued)

Name of shrimp/ lectin identified	Agglutination and binding specificity to erythrocytes/ glycoproteins/ sugars/microbes	Purification strategy and molecular weight of the lectin	Biological significance	Reference
Metapenaeus monoceros				
MmLec	Human erythrocytes and yeast *Saccharomyces cerevisiae* Arabinose, mannose, fucose, dextrose, rhamnose, N-acetyl glucosamine, and N-acetyl galactosamine	Mannose coupled sepharose CL-4B affinity chromatography 80 kDa	Antimicrobial activity—*Vibrio parahaemolyticus* and *Aeromonas hydrophila* and *Staphylococcus aureus* and *Enterococcus faecalis* Anticancerous activity against breast cancer cell line MDA-MB-231 and antibiofilm property	Preetham et al. (2019)
Metapenaeus dobsoni				
Md-Lec	Human erythrocytes and yeast *Saccharomyces cerevisiae* Fish pathogenic bacteria *Vibrio parahaemolyticus* and *Aeromonas hydrophila*	Mannose coupled sepharose CL-4B column 68 kDa	Antiviral potency against CyHV-2 and anticancerous activity of Md-Lec with breast cancer cell line, MDA-MB-231	Rubeena and Preetham (2019) and Rubeena et al. (2020)

Table 7.2 Shrimp lectin: Gene expression and function

Species/name of lectin	Gene expression/ function of lectin	Reference
Procambarus clarkii—PcLT (C-type lectin)	Expression of PcLT gene in hepatopancreas rPcLT—increase in mRNA expression in hemolymph phagocytosis and clearance of *V. alginolyticus* and WSSV	Chen et al. (2013)
P. monodon—two isoforms of Tachylectin5 like genes (PmTL5)	First PmTL5 isoform—expressed in the hindgut when treated with *V. harveyi* Second PmTL5-expressed in intestine and hemocytes	Soonthornchai et al. (2010)
Penaeus monodon—PmCLec (C-type lectin gene)	Recombinant PmCLec agglutinated *Staphylococcus aureus, S. haemolyticus* and *E. coli*	Wongpanya et al. (2016)
Penaeus monodon—PmFREP (recombinant fibrinogen-related lectin)	Expressed in insect cells -yielded a protein that can agglutinate red blood cells and *Pseudomonas aeruginosa*	Singrang et al. (2021)
Penaeus monodon—PmLec	PmLec—recombinant protein expressed in *E. coli* Hemagglutinating and opsonic properties	Luo et al. (2006)
Marsupenaeus japonicus—MjFREP1 (fibrinogen-related protein gene)	Expressed in the gills and the expression is induced by challenging with *vibrio anguillarum, Staphylococcus aureus*, or white spot syndrome virus Recombinant MjFREP1 fibrinogen-like domain agglutinated gram-positive bacteria like *Bacillus subtilis*, *Bacillus thuringiensis, Bacillus*	Chai et al. (2011)

(continued)

Table 7.2 (continued)

Species/name of lectin	Gene expression/ function of lectin	Reference
	megaterium, and *S. aureus*	
Marsupenaeus japonicus—MjFREP2	cDNA of FREP (MjFREP2)—*V. anguillarum* and *Staphylococcus aureus* challenge upregulated the MjFREP2 in the hemocyte induced phagocytosis	Sun et al. (2014)
Marsupenaeus japonicus—MjLTL1 (L-type lectin)	MjLTL1 silencing led to inhibition of clearance of *V. alginolyticus*	Wang et al. (2014)
Marsupenaeus japonicus—MjCTL	Cloned MjCTL was expressed in hepatopancreas, hemocytes, gill, stomach, intestine, heart, muscle and eyestalk Antibacterial activity against *V. parahaemolyticus*	Zheng et al. (2020)
Marsupenaeus japonicus—CTL33	Silencing of CTL33 expression led to intestinal dysbiosis, tissue damage and shrimp death Biofilm formation of CTL33 promoted intestinal bacteria and regulated the homeostasis of the microbiota	Zhang et al. (2021a, b)
Fenneropenaeus chinensis—Fclectin (C- type lectin)	Upregulation of Fc lectin expression after infection with bacteria/ LPS or WSSV	Liu et al. (2007)
Fenneropenaeus chinensis—Fc-hsL (hepatopancreas-specific calcium dependent C-type lectin)	Recombinant *Fc-hsL* agglutinated gram-positive, gram-negative bacteria and fungi	Sun et al. (2008)
Fenneropenaeus chinensis–FcCTL (C-type lectin-like protein)	rFcCTL Antimicrobial activity against gram-positive bacteria, gram-negative bacteria and fungi	Lai et al. (2013)

(continued)

Table 7.2 (continued)

Species/name of lectin	Gene expression/ function of lectin	Reference
Litopenaeus vannamei—LvLT	LvLT expressed only in hepatopancreas	Ma et al. (2007)
Litopenaeus vannamei—LvLec (C-type lectin gene)	*rLvLec* Agglutinated bacteria *E. coli* JM109	Zhang et al. (2009a, b)
Litopenaeus vannamei—*LvCTL1* (C-type lectin)	*LvCTL1* mRNA expressed in the hepatopancreas of *L. vannamei* Affinity for WSSV	Zhao et al. (2009)
Litopenaeus vannamei—*LvCTL1*and *LvCTL 2* (C-type lectin)	*LvCTL-br 1* expression induced in shrimp gill upon IHHNV infection	Costa et al. (2011)
Litopenaeus vannamei—LvLec1 (C-type lectin gene)	LvLec1 expressed in hepatopancreas LvLec1 transcripts altered in the hepatopancreas when challenged with LPS, *Micrococcus lysodeikticus,* and WSSV	Luo et al. (2011)
Litopenaeus vannamei—*LvLectin-1* and *LvLectin-2* (C-type lectins)	*L. anguillarum* challenge induced expression of both C-type lectins	Wei et al. (2012)
Litopenaeus vannamei—LvCTL3 (C-type lectin)	Expression of LvCTL3 in gills on challenge with lipopolysaccharides, *Vibrio parahaemolyticus,* and WSSV	Li et al. (2014)
Litopenaeus vannamei—LvGal (galectin)	Recombinant LvGal (rLvGal) was capable of binding with *V. anguillarum* and *Micrococcus lysodeikticus*	Hou et al. (2015)
Litopeneus vannamei—LvLTLC1 (L-type lectin)	rLvLTLC1 enhanced the clearance of *V. harveyi* following injection in shrimp, indicating LvLTLC1 as an opsonin	Tian et al. (2018)

(continued)

Table 7.2 (continued)

Species/name of lectin	Gene expression/ function of lectin	Reference
Litopenaeus vannamei—LvCTL5 (C-type lectin)	The recombinant LvCTL5 expressed in hepatopancreas silencing of the LvCTL5 gene in vivo could affect expression of some immune effector genes	Luo et al. (2019)
Litopenaeus vannamei—LdlrCTL	Silencing of LdlrCTL led to infection of shrimp by *Vibrio parahaemolyticus* but inhibited infection by WSSV	Liang et al. (2019)
Litopenaeus vannamei—LvCTL5 (C-type lectin)	rLvCTLs protein purified from *E. coli* Agglutinating activity against bacteria and fungi in vitro	Luo et al. (2019)
Litopenaeus vannamei—LvPLP (C-type lectin)	LvPLP expressed in hemocytes, hemolymph, heart and gills rLvPLP strongly agglutinated *S. aureus*, *Bacillus subtilis*, *V. parahaemolyticus,* and *V. anguillarum*	Bi et al. (2020)
Litopenaeus stylirostris—Ls-Stylicin 1	Recombinant *Ls-Stylicin 1* antifungal activity against the fungus, *Fusarium oxysporium*	Rolland et al. (2010)
Fenneropenaeus merguiensis—FmLC3 (C-type lectin)	Recombinant FmLC3 agglutinated bacterial strains and inhibited the microbial growth in vitro	Runsaeng et al. (2017)
Fenneropenaeus merguiensis—FmLC6 (C-type lectin)	The transcription of FmLC6 was detected in hepatopancreas Purified rFmLC6 induced microbial agglutination in the presence of calcium	Runsaeng et al. (2018)

7.5.1 *Crayfish:* Procambarus clarkii, Pacifastacus leniusculus

Natural agglutinins were identified from the Crayfish *Procambarus clarkii* that agglutinated marine bacteria, chicken, and rabbit erythrocytes (Miller et al. 1972). The agglutinin was thermolabile, pH sensitive at high acidic, and alkaline pH with molecular weight greater than 150 kDa. A 420 kDa lectin with a high specific activity against trypsinized rabbit erythrocytes was purified from the plasma of *Pacifastacus leniusculus*. Sialoglycoproteins PSM, BSM, fetuin, and LPS from *E. coli* K 235 highly inhibited the agglutinin activity (Kopacek et al. 1993). Chen et al. (2013) purified a lectin, PcLT from crayfish, *Procambarus clarkii* that was capable of binding to *Vibrio alginolyticus* and WSSV. RT-PCR analysis revealed expression of PcLT in hepatopancreas and there was an increase in mRNA after challenge with *Vibrio alginolyticus* and WSSV. There was also a considerable augment in mRNA expression and function of immune factors in the hemolymph like peroxinectin, phenoloxidase, and superoxide dismutase in response to rPcLT. Increased phagocytosis and the clearance of *V. alginolyticus* and WSSV suggests the role of PcLT as a pathogen recognition molecule involved in host defense against pathogens.

7.5.2 *Black Tiger Prawn:* Penaeus monodon

A lectin that has affinity towards sialic acid was isolated from hemolymph of *P. monodon* using a fetuin agarose column by Ratanapo and Chulavatnatol (1990). The purified lectin showed a major peak on superose 12 column with a molecular weight of 420 kDa. Monodin agglutinated *Vibrio vulnificus*, a bacterium which causes disease among prawns. The hemolymph lectin was purified on FPLC anion exchange chromatography using mono–Q column (Ratanapo and Chulavatnatol 1992) and a 27 kDa lectin was obtained. Induction of heat killed *Vibrio alginolyticus* into *Penaeus monodon* caused an increase in induced lectins within 1 day post challenge and the presence of lectins was observed for a short time when compared to bactericidins. Hemagglutinin activity was detected against sheep RBC by the induced lectins (Adams 1991).

Tachylectin5 like genes (PmTL5) and its isoforms were identified in *P. monodon* by Soonthornchai et al. (2010). The first PmTL5 isoform was expressed in the hindgut and the second in the intestine and hemocytes when treated with *V. harveyi*. Wongpanya et al. (2016) cloned and characterized a CTL gene, PmCLec from *Penaeus monodon*. The purified recombinant PmCLec agglutinated *Staphylococcus aureus, S. haemolyticus,* and *E. coli*, suggesting that PmCLec functions as a PRR that is of concern in shrimp immunity.

Biochemical and structural analysis of recombinant fibrinogen-related lectin (PmFREP) from *P. monodon* expressed in insect cells had yielded a protein that

can agglutinate red blood cells (Singrang et al. 2021). PmFREP binds to N-acetyl sugars in the presence of calcium and agglutinated *P. aeruginosa* but failed to agglutinate the shrimp pathogen *V. parahaemolyticus*. A CTL (PmLec) specific to bacterial LPS was isolated from the shrimp *Penaeus monodon* which exhibited hemagglutination, bacterial agglutination as well as opsonic properties. Functioning of lipopolysaccharide-binding lectin as an opsonin in enhancing hemocytic phagocytosis and its affinity to bind to Gram-negative bacteria was reported by Luo et al. (2006). PmLec cDNA sequence was first documented from this shrimp and the recombinant protein expressed in *E. coli* showed similar hemagglutinating and opsonic properties as that of native protein.

7.5.3 *Kuruma Shrimp:* Penaeus japonicus

A sheep erythrocyte specific lectin with hemagglutinating and opsonic activity was documented from the hemolymph of *Penaeus japonicus* (Kondo et al. 1992). Two lectins calcium independent and calcium dependent were identified and the hemagglutinating activity was inhibited by D-idose, D-mannosamine, and NANA. Presence of multiple lectins in *P. japonicus* was confirmed by Muramoto et al. (1995). The lectin was initially purified using a PSM—sepharose 6-B column followed by FPLC on a Mono Q Column. Four active fractions with specificity to different mammalian erythrocytes and glycoproteins proved the heterogeneity of the lectin. Characterization of L3 of *P. japonicus* was performed by Shanon et al. (2002) and it was observed that the lectin had strong affinity towards rat and mice erythrocytes, thermostable up to 56 °C with complete loss of activity at 70 °C to 80 °C, and stable at pH between 8 and 9. The hemagglutination was strongly inhibited by galactose, mannose, arabinose, glucosamine, and GluNAc with high agglutination titer against bacteria *V. alginolyticus* HW283. Hemagglutinating activity was found in the serum and muscular extract of *P. japonicus* (Chen and Congjie 2002) and physico-chemical analysis revealed that different lectins were present both in serum and muscle extract. The serum agglutinin was calcium dependent, stable between pH 4.0 and 7.0 and temperature up to 90 °C while the lectin isolated from the muscle was calcium independent, stable between pH 6.0 and 7.0, temperature 4–80 °C.

A naturally occurring calcium independent lectin *PjLec,* with a molecular weight of 37 kDa was reported from the haemolymph of *Penaeus japonicas* (Yang et al. 2007). The lectin was purified on fetuin-Sepharose affinity column and the purified lectin agglutinated mammalian erythrocytes and bacteria. The agglutination of the purified lectin was inhibited by ManNAc, Neu5Ac, lipopolysaccharides, and Bovine submaxillary mucin. Gao et al. (2020) identified and characterized a novel mannose-binding CTL (PjLec2) from the hemolymph and hepatopancreas of marine shrimp *Penaeus japonicus* which agglutinated *V. alginolyticus* and *Aeromonas hydrophila*. The CRD of PjLec2 has an EPN (Glu-Pro-Asn) motif that can recognize mannose and act as a PRR in shrimp.

7.5.4 *Kuruma Shrimp:* Marsupenaeus japonicus

A fibrinogen-related protein gene (MjFREP1) that encodes a protein of 270 amino acids and a fibrinogen-like domain (223 amino acids) was isolated from *M. japonicus* by Chai et al. (2011). This gene expression was observed in gills followed by enhanced immune response on challenge with *V. anguillarum, S. aureus*, or WSSV. The recombinant MjFREP1 fibrinogen-like domain agglutinated *B. subtilis*, *B. thuringiensis, B. megaterium,* and *S. aureus* and it required calcium for its agglutination. The fibrinogen like domain of MjFREP1 was capable of binding to peptidoglycans, LPS, bacteria, and WSSV suggesting its role in immune response against different pathogens. An L-type lectin MjLTL1 was reported from the kuruma shrimp, *Marsupenaeus japonicus* by Xu et al. (2014). This lectin was found to have a leguminous lectin domain and transmembrane region. When the shrimps were challenged with *V. alginolyticus*, there was an upregulation of MjLTL1 and the lectin agglutinated bacteria through lipopolysaccharide and peptidoglycan binding. MjLTL1 enhanced the elimination of *V. alginolyticus* in vivo and this was inhibited by MjLTL1 silencing.

A cDNA of FREP (MjFREP2) was recognized from *M. japonicas* by Sun et al. (2014). This MjFREP2 contained 1138 bp with an ORF of 954 bp that encodes a 317 amino acid protein and comprise a signal peptide and fibrinogen-like domain. MjFREP2 was observed in hemocytes, heart, hepatopancreas, gills, stomach, and intestine. During *V. anguillarum* and *Staphylococcus aureus* challenge, the MjFREP2 was upregulated in the hemocytes. Immunocytochemical studies revealed that initially the MjFREP2 was present in the cytoplasm of hemocytes but after challenge with *V. anguillarum* and *Staphylococcus aureus*, the MjFREP2 was transported to the membrane or secreted out of the cell. The secreted MjFREP2 bound to the bacteria and induced phagocytosis. Alenton et al. (2019) suggested the presence of endogenous MjGCTL (nMjHCTL) in the gills of *Marsupenaeus japonicus*. This lectin exhibited in vivo bacterial phagocytosis by hemocytes.

L-type lectin was purified and characterized from shrimp *Marsupenaeus japonicus* (Xu et al. 2020). The lectin designated as MjLTL2 was observed in hemocytes, heart, hepatopancreas, gill, stomach, and intestine. MjLTL2 was upregulated upon injection of *V. anguillarum* and WSSV in shrimp. The calcium independent MjLTL2 agglutinated bacteria and it bound specifically to lipopolysaccharide and peptidoglycan. Wang et al. (2014) reported a CTL, *MjHeCL* from *M. japonicus* that can reduce the hemolymph microbes by inhibiting bacterial multiplication by altering the AMPs. Zheng et al. (2020) characterized a novel CTL and it was cloned with *M. japonicus* (MjCTL). It consists of 513 bp that encodes a polypeptide of 170 amino acids, a signal peptide and a CRD specific for CTLs. MjCTL was observed in diverse tissues like hepatopancreas, hemocytes, gill, stomach, intestine, heart, muscle, and eyestalk. In addition, MjCTL revealed antibacterial potency against *V. parahaemolyticus*, suggesting that MjCTL functions as a PRR. A digestive tract specific CTL33 was identified in the intestine of the kuruma shrimp *Marsupenaeus japonicus* by Zhang et al. (2021a, b). Silencing of

CTL33 caused intestinal dysbiosis, tissue injury and mortality of shrimps. The biofilm formation of CTL33 promoted the establishment of bacteria in intestine and regulated the homeostasis of the microbes.

7.5.5 *Blue Shrimp:* P. stylirostris, *Brown Shrimp*—P. californiensis *and Rose Shrimp* P. longirostris

Vargas-Albores et al. (1992) screened the hemolymph of blue shrimp, *P. stylirostris* for the presence of agglutinins. The agglutinin agglutinated mammalian erythrocytes and the activity decreased with decrease in size. The team in 1993 reported the presence of agglutinin in brown shrimp *P. californiensis*. The lectin was purified on a GalNAc agarose column and it yielded two fractions which agglutinated mouse erythrocytes (fraction A) and both human and mouse erythrocytes (fraction B). Brown shrimp hemagglutinin (BSH-1: fraction-B) was purified using Sepharose 6B column and a 180 kDa lectin was obtained. Hemagglutination against mammalian erythrocytes was detected in the sea prawn, *P. longirostris* (Fragkiadakis and Stratakis 1995). Two lectins and an agglutinin were purified by adsorption on formalinized rabbit erythrocytes. Of the two cation-dependent lectins, 27 kDa lectin was specific to NeuAc and galactose and the 36 kDa was specific to GalNAc. Both the lectin and the agglutinin strongly agglutinated *Pseudomonas aeruginosa*.

7.5.6 *Indian White Prawn:* P. indicus

Human A erythrocyte specific lectin which was cation independent, thermostable up to 80 °C and active between pH 3 and 9 was isolated from the marine prawn *P. indicus* (Maheswari et al. 1997). The serum agglutinin was highly specific for acetylated aminosugars. Jayasree (2001) isolated a lectin from the hemolymph of *P. indicus* by gel filtration chromatography. The molecular weight of the agglutinin was deduced as 181 kDa. The proteinaceous nature of the agglutinin was confirmed by treatment with denaturing agents TCA, phenol, chloroform and 45% ammonium sulfate. A 200 kDa *Fenneropenaeus* (*Penaeus*) *indicus* lectin (FIA) was purified by affinity chromatography on GluNAc Sepharose 6B column (Maheswari et al. 2002). The hemagglutinating activity of the agglutinin was cation independent and insensitive to their chelators. FIA agglutinated bacteria isolated from infected shrimps and the antimicrobial action was inhibited by LPS obtained from Gram negative bacteria.

7.5.7 *Chinese Shrimp,* Fenneropenaeus chinensis

A C-type lectin was cloned from the hemocytes of the Chinese shrimp *F. chinensis* (Liu et al. 2007). The immune response was evident from the expression profiles of the Fc lectin gene upon bacterial / LPS challenge. Novel calcium dependent CTL, *Fc-hsL* was reported from the hepatopancreas of *F. chinensis* (Sun et al. 2008). The cDNA of *Fc-hsL* is 571 bp long with a 480 bp open reading frame that encodes a 159-residue protein. The lectin has an EPN (Glu-Pro-Asn) motif that can bind to a site specific for mannose. This lectin was found in the hemolymph and the hepatopancreas of bacteria and virus challenged shrimps. Recombinant *Fc-hsL* exhibited agglutinating activity against bacteria and fungi. Lai et al. (2013) identified and characterized the C-type lectin-like protein from the Chinese shrimp, *F. chinensis* named as FcCTL. It showed that the full-length cDNA of 859 bp consists of 220 aminoacids with one CRD. The purified rFcCTL demonstrated antimicrobial activity against bacteria and fungi. Agglutinating activity of rFcCTL was totally inhibited by GlcNAc, LPS, D-galactose, and maltose.

7.5.8 *Green Tiger Shrimp,* Penaeus semisulcatus *and Sao Paulo Shrimp,* P. paulensis

A 37 and 118 kDa lectin (*Ps*-Lec) was purified and characterized from *Penaeus semisulcatus* by affinity column chromatography with mannose coupled Sepharose CL-4B column (Sivakamavalli and Vaseeharan 2014). The surface morphology of *Ps*-Lec exhibited crystalline nature, as evident by TEM analysis. *Ps*-Lec agglutinated several vertebrate erythrocytes and few Gram-negative and Gram-positive bacterial cells and fungal species, *Candida albicans*. Preetham et al. (2020) studied the antibiofilm and immunological properties of a 66 kDa lectin (*Semisulcatus*-lectin) obtained from shrimp *Penaeus semisulcatus*. *Semisulcatus*-lectin agglutinated yeast *Saccharomyces cerevisiae* and several human erythrocytes and displayed antibacterial activity against *V. parahaemolyticus* and *Aeromonas hydrophila*, suggesting a potential role in phagocytic activities. An 153 kDa lectin was isolated from the hemolymph of *P. paulensis* using fetuin agarose affinity column followed by gel filtration on superose 12 (Marques and Barracco 2000).

7.5.9 *White Shrimp:* Litopenaeus setiferus*;* L. schmitti*;* L. vannamei *and Blue Shrimp:* Litopenaeus stylirostris

Alpuche et al. (2005) purified a 291 kDa lectin (LsL) from the hemolymph of *Litopenaeus setiferus* by affinity chromatography on gluteraldehyde fixed stroma

from rabbit erythrocytes. The lectin agglutinated guinea pig and rabbit erythrocytes and the hemagglutination was inhibited by sialylated O-glycosylated proteins and N-acetylated sugars.

A lectin specific for sialic acid, and O-sialoconjugates and LPS was purified by Cominetti et al. (2002) from the hemolymph of *Litopenaeus schmitti*. The agglutinin was purified by affinity chromatography on fetuin—agarose column and a 220 kDa band with two subunits of 31 and 34 kDa was observed on SDS PAGE. The purified lectin was calcium independent and was inhibited by acetylated sugars, in particular sialic acid (Neu5Ac) and O-sialoglycoconjugates (BSM).

The presence of CTL in the hepatopancreas of *Litopenaeus vannamei* was suggested by Gross et al. (2001). The white leg shrimp, *Litopenaeus vannamei* showed maximum agglutinability with rabbit erythrocytes and the HA activity was inhibited by simple sugars galactose, mannose, fructose, and glucose (Leung et al. 2005). The lectin was calcium dependent, stable at pH 4.0, and thermosensitive. Sun et al. (2007) purified and characterized a calcium dependent serum lectin, *LVL* from *Litopenaeus vannamei* using fetuin-coupled agarose affinity chromatography. This lectin agglutinated human A, B, O, mouse, and chicken erythrocytes. The agglutinating activity of the hemolymph was inhibited by GalNAc, GluNAc, and NeuAc, glycoproteins BSM and fetuin. The lectin had a molecular weight of 172 kDa consisting of 32 and 38 kDa subunits. This lectin also agglutinated diverse Gram-negative bacteria. Ma et al. (2007) cloned and characterized the lectin-like cDNA (LvLT) of *L. vannamei* which consists of 1035 bp that encodes for a protein with 345 amino acids and was designated as LvLT cDNA. It has two CRDs that bound galactose and mannose, respectively. RT-PCR analysis revealed that LvLT is expressed only in hepatopancreas and the expression level of LvLT declined in the first 2 h and later increased after 4 h, when juvenile shrimps were injected with shrimp extracts containing WSSV.

A CTL gene LvLec was cloned and characterized from the hemocytes of *Litopenaeus vannamei* by Zhang et al. (2009a, b). The cDNA was 618 bp with a 5′ and 3′ terminal UTR of 60 bp and 87 bp and a poly (A) tail. The *rLvLec* showed agglutination activity against the bacteria *E. coli* JM109 which was calcium dependent and inhibited by sugar mannose and chelating agent EDTA. A novel C-type lectin, *LvCTL1* formed of 156 residue polypeptides with a C-type CRD, an EPN motif and specificity for mannose was reported from *L. vannamei* by Zhao et al. (2009). *LvCTLI* mRNA expression was reported from hepatopancreas and the r*LvCTL1* showed hemagglutination activity and specificity for mannose and glucose similar to *LvCTL1*. It exhibited anti-white spot syndrome virus activity by interacting with envelope proteins of WSSV.

Two CTL precursors *LvCTL-br 1* and *-br 2* were cloned from *L. vannamei* with cDNAs having ORFs of 1044 nucleotides that encodes two identical domain polypeptides of 347 residues and recognized galactose in CRD1 and a mutated mannose-binding site in CRD2 (Costa et al. 2011). IHHNV infection of shrimps revealed the expression of *LvCTL-br 1* in gills as evident through RT-PCR. Molecular modeling of *LvCTL-br1* and *-br2* showed three amino acid substitutions in CRD1 and a long loop of *LvCTL-br1* CRD2 that can accommodate intricate saccharides (Costa et al.

2011). A C-type hepatopancreas specific lectin gene (LvLec1) with an ORF of 510 bp that encodes 169 amino acids was cloned from *L. vannamei* by Luo et al. (2011). Expression of LvLec1 transcripts in the hepatopancreas was altered following artificial challenge with LPS, *Micrococcus lysodeikticus,* and WSSV. LvLec1 mediated pathogen recognition and played an important part in the clearance of pathogens. Wei et al. (2012) reported two CTLs—*LvLectin-1* and *LvLectin-2* from *L. vannamei*. The cDNA of the two CTLS was 567 and 625 bp with an ORF of 471 and 489 bp. Both the CTLs encoded a single CRD and the QPN (Gln^{122}-Pro^{123}-Asn^{124}) in *LvLectin-1* but QPD (Gln^{128}-Pro^{129}-Asp^{130}) in *LvLectin-2* motif of calcium binding site 2 determined its specific sugar binding site. The two lectins were mostly expressed in hepatopancreas and hemocytes while the expression of two CTL mRNA in hemocytes drastically changed following challenge with *Listonella anguillarum* or WSSV. Induction of *L. anguillarum*, upregulated the expression of both CTLs when compared to the control group, and an increase of *LvLectin-1* was noted when compared to *LvLectin-2*. On the other hand, the expression of *LvLectin-2* was significantly upregulated when stimulated with WSSV but the expression of *LvLectin-1* was downregulated post-stimulation. A C-type lectin was identified from pacific white shrimp *L. vannamei* (LvCTL3) by Li et al. (2014) that has a single CTLD. LvCTL3 responded to bacterial, viral, and immune challenges following injection of *V. parahaemolyticus* and WSSV. The rLvCTL3 protein expression was noted in *E. coli* and it was isolated by Ni-affinity chromatography. The purified calcium dependent LvCTL3 agglutinated *V. alginolyticus, V. parahaemolyticus, B. subtilis*. The agglutination of LvCTL3 was completely abolished after chelation with EDTA, suggesting its calcium dependency. In vivo bacterial challenging experiments indicated LvCTL3 play a momentous role in the shrimp defense against pathogenic infection. Hou et al. (2015) reported the presence of galectin (LvGal) cDNA with an ORF of 1017 bp that encodes a protein of 338 amino acids in *Litopenaeus vannamei***.** This galectin was found to have a CRD and amino acid residues involved in dimerization. When the shrimps were challenged with *V. anguillarum* there was an upregulation of mRNA in gills and hemocytes. The recombinant LvGal (rLvGal) was capable of binding with *V. anguillarum* and *Micrococcus lysodeikticus*. In vivo experiments revealed the role of hemocytes in phagocytosis.

A FREP-like homolog was identified in *Litopenaeus vannamei* (LvFrep) by Coelho et al. (2016). The sequence of LvFrep revealed a fibrinogen-related domain (FReD) that was similar to FREP-like proteins observed in few invertebrates and to ficolins from crustaceans. The LvFrep was expressed only in hemocytes during *Vibrio* infection. Presence of LvFrep transcripts in the early fertilized eggs suggests the involvement of this gene in the antimicrobial defense during shrimp development. Tian et al. (2018) reported a new L-type lectin (LvLTLC1) from *Litopeneus vannamei*. The cDNA is 1184 bp with an ORF of 990 bp that encodes a protein of 329 amino acids and has L-type lectin like domain and a transmembrane helix region. Phylogenetic analysis documented that LvLTLC1 belonged to VIP36-like family and was observed in all tested tissues but gills and hepatopancreas showed higher activity. It was upregulated following immune challenge against *V. harveyi*

and LPS. The agglutinating activity of rLvLTLC1 was expressed in *S. aureus*, *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. cholera*, *V. vulnificus*, *Pseudomonas aeruginosa,* and *P. fluorescens* in the presence of calcium. rLvLTLC1 enhanced the clearance of *V. harveyi* following injection in shrimps, indicating LvLTLC1 as an opsonin.

A new CTL gene LvCTL5 expressed in hepatopancreas and upregulated on challenge with pathogens was documented from *Litopenaeus vannamei* by Luo et al. (2019). When the LvCTL5 gene was silenced in vivo, immune effector genes was less expressed and the phagocytic action of hemocytes was decreased. Song et al. (2019) cloned a CTL gene (LvCTLU) from *L. vannamei* which was responsible for microbial agglutination and phagocytosis. Bacterial agglutination was calcium dependent and downregulation of the gene expression increased the mortality of shrimps infected with *V. haemolyticus*.

A LdlrCTL was identified from *Litopenaeus vannamei* by Liang et al. (2019) which after immune stimulation was highly expressed in hemocytes. Silencing of LdlrCTL led to infection of shrimp by *V. parahaemolyticus* but inhibited infection by WSSV. LdlrCTL showed agglutination activity against bacteria and fungi and enhanced phagocytosis by hemocytes. Immune effector genes were expressed and signaling pathway mechanism was altered in LdlrCTL-silenced shrimp, showing the role of LdlrCTL in immune regulation. Luo et al. (2019) identified a novel CTL (LvCTL5) in *Litopenaeus vannamei* that was highly expressed in hepatopancreas following infection with pathogenic microbes. The rLvCTLs protein purified from *E. coli* agglutinated bacteria and fungi in vitro.

A perlucin-like protein (PLP), identified as a CTL, was observed in the cDNA of *L. vannamei*. LvPLP has a 540 bp with a single CRD and a ORF that encodes a protein of 179 amino acids. PCR analysis revealed the presence of LvPLP in hemocytes, hemolymph, heart, and gills. rLvPLP has the capacity to bind directly to LPS and peptidoglycan with differing avidity, and it was able to bind strongly to *S. aureus*, *Bacillus subtilis*, *V. parahaemolyticus,* and *V. anguillarum*. The bacterial agglutinating activity observed in *V. parahaemolyticus* and *V. anguillarum* shows that *LvPLP* has a key role in *L. vannamei* immunity (Bi et al. 2020).

Rolland et al. (2010) characterized an AMP *Ls-Stylicin 1* from *Litopenaeus stylirostris* which consists of 82 residues, negatively charged with a proline-rich N and C-terminal region consisting of 13 cysteine residues. The recombinant *Ls-Stylicin 1* separated as monomeric and dimeric forms exhibited antifungal activity against the fungus, *Fusarium oxysporium*. The *Ls-stylicin* was also capable of agglutinating *Vibrio penaeidae* in vitro.

7.5.10 Banana Shrimp: Fenneropenaeus merguinensis

Presence of a 316.2 kDa lectin (FML) in the hemolymph of *F. merguinensis* was first documented and purified using fetuin agarose column by Rittidach et al. (2007). Rabbit and rat erythrocyte were agglutinated by FML and was specific towards

NeuAc. Utarabhand et al. (2007) reported the presence of agglutinating activity of a calcium dependent lectin from the hemolymph of *P. merguiensis* that agglutinated rabbit erythrocytes. The lectin was specific for sugar NeuNAc and hemagglutination activity was inhibited by glycoproteins PSM and fetuin. FML agglutinated *Vibrio harveyi*, *Vibrio parahemolyticus,* and *Vibrio vulnificus*.

A new mannose-specific CTL (FmLC3) was identified from *F. merguiensis* hepatopancreas by RT-PCR and 5′ and 3′ RACE (Runsaeng et al. 2017). The recombinant FmLC3 agglutinated different bacterial strains in a calcium dependent manner and it inhibited the microbial growth in vitro. Senghoi et al. (2017) identified a CTL gene—FmLC5 from *F. merguiensis*. FmLC5 consisted of 1526 bp and ORF of 852 bp that encoded a 284 amino acid peptide with a molecular mass of 31.47 kDa, two CRDs, with a calcium binding site-2 and a QPD motif for galactose-binding in each CRD.

FmLC4 a CTL was cloned from the hepatopancreas of *F. merguiensis* by RT-PCR and 5′ and 3′ rapid amplification of cDNA ends (Utarabhand et al. 2017), FmLC4 consists of 706 bp with an ORF of 552 bp that encodes a peptide of 184 amino acids. The molecular mass of 20.4 kDa FmLC4 cDNA includes a signal peptide of 19 amino acids, isoelectric point of 5.13 and a single CRD with a QPD motif and calcium binding site, suggesting FmLC4 role as a defensive molecule.

Runsaeng et al. (2018) identified a CTL-FmLC6 with dual CRD, and a motif from the hepatopancreas of shrimp *F. merguiensis*. Transcription of FmLC6 was noticed in hepatopancreas and its immunostimulant property was confirmed after challenge with pathogens. The expression of FmLC6 increased steadily and reached the peak at 12 h post-injection. Purified rFmLC6 of an entire ORF and two CRDs of FmLC6 formed in *E. coli*, induced microbial agglutination in the presence of calcium. The agglutination was induced by rFmLC6, rCRD1, and rCRD2 and was inhibited by combination of galactose and mannose in a dose-dependent manner. Purified rFmLC6 was able to bind to WSSV particles suggesting FmLC6 as a PRR and to mediate the response of shrimp to the pathogens.

A distinctive CTL, FmLFd was identified in the hemocytes of *F. merguiensis*. FmLFd had one ORF that encodes a peptide of 312 amino acids and a signal peptide of 18 amino acids. Analysis of FmLFd revealed that it contained a fibrinogen-like domain with a Ca^{2+}- binding region and affinity towards N-acetyl glucosamine. Immunochallenge with *V. parahaemolyticus* and *V. harveyi*, documented an increase in the expression of FmLFd suggesting it as a PRR in shrimp immune defense against bacterial and viral pathogens (Senghoi et al. 2019).

7.5.11 Speckled Shrimp: Metapenaeus monoceros

An 80 kDa lectin MmLec was reported from the hemolymph of *M. monoceros* and isolated using mannose coupled sepharose CL-4B affinity chromatography (Preetham et al. 2019). MmLec agglutinated human erythrocytes and *Saccharomyces cerevisiae*. This lectin has affinity to bind with sugars such as arabinose,

mannose, fucose, dextrose, rhamnose, GluNAc, and GalNAc. Antimicrobial activity was observed in MmLec against bacteria *V. parahaemolyticus*, *A. hydrophila*, *S. aureus,* and *E. faecalis*. MmLec also exhibited anticancerous property as evident with breast cancer cell line MDA-MB-231 and antibiofilm property towards the bacterial species treated at different concentrations of MmLec. The lectin was capable of stimulating encapsulation of Sepharose beads by haemocytes and was capable of reducing the biofilm formed by the tested bacteria.

7.5.12 Flower Tail Prawn: Brown Shrimp, Metapenaeus dobsoni

Lectin, Md-Lec has been identified and purified from the hemolymph of brown shrimp *Metapenaeus dobsoni* (Rubeena and Preetham 2019) by affinity chromatography with mannose coupled sepharose CL-4B column. Its molecular mass was identified as 68 kDa on SDS-PAGE and confirmed by MALDI-TOF. The presence of functional groups was analyzed by FTIR. Md-Lec agglutinated human erythrocytes and yeast *Saccharomyces cerevisiae* as observed on light microscopy. Md-Lec also agglutinated fish pathogenic bacteria *Vibrio parahaemolyticus* and *Aeromonas hydrophila*. Antiviral potency was observed against CyHV-2 and anticancerous activity of Md-Lec was noted with breast cancer cell line, MDA-MB-231. Md-Lec conjugated copper nanoparticles (lectin-pCuS NPs) was introduced into the bacteria infected fish Nile tilapia (*Oreochromis niloticus*) and the results revealed that the fish fully recovered from the infection, indicating the effectiveness of the complex (lectin-pCuS NPs) than the individual molecules (Rubeena et al. 2020).

7.6 Lectins in Freshwater Prawn

7.6.1 Macrobrachium rosenbergii

Humoral lectins have been identified and characterized from freshwater prawns. The genus *Macrobrachium* was identified to be rich source of lectins that are specific to N acetylated sugars, sialic acid and capable of agglutinating several erythrocytes and bacterial species. Vasta et al. (1983) isolated multiple lectins from the freshwater prawn, *Macrobrachium rosenbergii* that agglutinated pronase treated mammalian erythrocytes, except for horse and rat erythrocyte.

A 9–0 acetyl sialic acid specific lectin from *M. rosenbergii* with a molecular weight of 19 kDa was purified on a rat stroma column affinity chromatography (Vazquez et al. 1993) and BSM Sepharose 4B column (Vazquez et al. 1994). The purified lectin was specific for rat and rabbit erythrocytes and it agglutinated selected strains of *Pasteurella multicoda*. Vazquez et al. (1997) identified a membrane lectin

on the hemocyte surface which showed specificity towards N-acetylated carbohydrates and sialylated glycoproteins. A dimeric glycoprotein (MrL) of 9.5 kDa specific for sialic acid, with affinity towards rat and rabbit erythrocytes and few bacterial strains was identified from the hemolymph of *M. rosenbergii* (Zenteno et al. 2000). The quantity of lectin present in the hemolymph of *M. rosenbergii* documented, highest concentration in post-larval prawns and lowest in molt stage adult animals. The N-acetylated sugars (GluNac, GalNAc, and NeuAc) inhibited the hemagglutinin activity and it was reported that the lectins may play role in transportation of acetylated sugars in juvenile prawns and hemagglutinating activity may be influenced by the developmental stages of prawns (Agundis et al. 2000).

Huang et al. (2016) reported a lectin *Mr*CTL from *M. rosenbergii* with a single CRD and an EPN motif (Glu-Pro-Asn). It consists of a 657 bp ORF encoding for a protein of 218 amino acids. MrCTL was highly expressed, after challenging with *Vibrio parahaemolyticus*. It agglutinated bacteria in the presence of calcium and rMrCTL bound to LPS and peptidoglycan in a dose-dependent manner and accelerated bacterial clearance. Snigdha et al. (2019) reported the expression of Mr.(T)Lec4 and Mr.(T)Lec2 in the hepatopancreas tissue of *M. rosenbergii* following challenge with *Vibrio harveyi* and *M. rosenbergii* noda virus (MrNV). 23- and 28-folds increase in activity was observed for Mr.(T)Lec4 and Mr.(T)Lec2, respectively, suggesting its role in the upregulation of immune response of *M. rosenbergii.*

A calcium dependent lectin was characterized from the hemolymph of *M. rosenbergii* by Jyotirmaya et al. (2020). It agglutinated rabbit RBCs and the agglutinin was sensitive to pH and temperature and hemagglutinating activity was repressed by NANA and fetuin. Purification of the lectin on rabbit erythrocyte stroma affinity column yielded a protein of MW 427 kDa on SDS-PAGE.

7.6.2 Macrobrachium nipponense

A novel 2-transmembrane CTL (Mn-2TM-cLec) with single CRD was obtained from *M. nipponense* (Huang et al. 2019). Mn-2TM-cLec was distributed in hemocytes, hepatopancreas and gills and significantly upregulated in gills on challenge with *S. aureus* and *V. parahaemolyticus*. The rCRD of Mn-2TM-cLec was able to bind to LPS, peptidoglycans, bacterial strains and it agglutinated *S. aureus* and *V. parahaemolyticus* in a calcium dependent manner, suggesting Mn-2TM-cLec as a PRR in prawn defense system.

7.6.3 Macrobrachium malcolmsonii

Indian river prawn, *M. malcolmsonii* was studied for the presence of agglutinins by Acharya et al. (2004). A 413 kDa GalNAc specific lectin was purified by affinity

chromatography and it yielded five submits on SDS PAGE. The lectin was stable at pH 3.0–7.0, temperature sensitive and calcium dependent.

7.6.4 Macrobrachium americanum

A lectin, *MaL* from the hemolymph of the prawn *Macrobrachium americanum* was purified and partially characterized by Pereyra et al. (2012). The purified lectin was formed of three subunits (86.6, 66.2, and 42.7 kDa). This lectin agglutinated rat erythrocytes and this activity was inhibited by NANA (sialic acid), GluNac, and GalNac. The lectin activity was also inhibited by sialylated fetuin and bovine submaxillary mucin. When the shrimps were exposed to stress there was an increase in lectin concentration and specific activity.

7.7 Biological Significance of Shrimp Lectins

The biological role of lectins has been documented in several reports. A significant feature of shrimp lectins is its capability to agglutinate mammalian erythrocytes, cells, and broad spectrum of bacteria and fungi (Xu et al. 2010; Zhang et al. 2009a). Opsonization, encapsulation, and clearance of microbes and parasites have been observed in shrimps (Wang et al. 2009b). Viral disease among shrimps is quite common and has created great loss to the aquaculture industry. Microbicidal activity has been reported from the shrimp lectin and shrimp CTLs play an important role in antiviral immunity. FcLec1 inhibited the growth of both bacteria and fungi (Sun et al. 2008) by destroying the bacterial cell wall (Yu et al. 2007). Five shrimp CTLs have been found to interact with the structural proteins of WSSV (Song et al. 2010; Zhao et al. 2009; Tsai et al. 2006). Similar observations were recorded with MjLec A, B, and C from Kuruma Shrimp (Song et al. 2010), LvCTl1 from *L. vannamei* which binds to WSSV virions (Zhao et al. 2009), and YHV (Yellow head virus) (Junkunlo et al. 2011).

7.8 Conclusion

Lectins are multivalent glycoproteins with an inherent capacity to identify and bind to the lectin ligands expressed on cell surface. Recognition and discrimination of self from non-self-molecules and elimination of foreign pathogens has been well defined in the immune system of shrimps. Genes responsible for the expression of the lectins and cloning of genes have created a new arena in the shrimp lectin research. Biomedical application of shrimp lectins in distinguishing microbes and cells

envisions a promising future for lectin targeted therapy and lectin nano-conjugated drug delivery.

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Conflict of Interest The authors declare that they have no conflict of interest.

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Chapter 8
Molecular Cloning and Functional Interaction by Computational Analysis

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Abstract Crustacean lectins are pattern recognition proteins which are deficient in an adaptive immune system and solely on their innate immunity. Insights into molecular level of lectin would help us to determine the crustacean immune regulation mechanism. Numerous lectins were found in crustaceans which play a role in the lectins identified and sequenced at the molecular and transcriptional levels. Some of these interactions implicate definite carbohydrate structures and proteins, notably lectins that identify and bind them specifically. It is generally known that lectin-carbohydrate interactions play a crucial function in the immune system, as they mediating and regulating the immune response interactions. Thus, more information on the lectin carbohydrate interaction is necessary to clarify their participation in the immune system and to learn more about how lectins regulate the immune response in the crustaceans relying on their carbohydrates specificity. This chapter analyses the molecular cloning and its functional interactions of crustacean lectins were explored.

Keywords Crustacean · Lectin · Innate immunity · Molecular cloning

Abbreviations

CRD	Carbohydrate recognition domain
CTLD	C-type lectin like-domain
FReD	Fibrinogen-related domain
FREPs	Fibrinogen-related proteins
Gal	Galactose

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GalNAc	*N*-acetylgalactosamine
GlcNAc	*N*-acetylglucosamine
LPS	Lipopolysaccharides
LTL	L-type lectins
Man	Mannose
ManNAc	*N*-acetylmannosamine
MBL	Mannose-binding lectin
N/D	No determination
NeuAc	N-acetylneuraminic acid
PAMPs	Pathogen associated molecular patterns
PRRs	Pattern recognition receptors
ProPO	Prophenoloxidase
WSSV	White Spot Syndrome Virus

8.1 Introduction

In invertebrates, crustacean possess a primordial form of immune system that attacks the invading pathogens, as they provide habitat in an environment rich in microbes and parasites. Due to the lack of an adaptive immune system, which relies merely on innate immunity to defend itself (Jayanthi et al. 2017). As the first action of innate immunity is mediated by PRRs (pattern recognition receptors), which can recognize on the cell surface and bound with conserved PAMPs (pathogen associated molecular patterns). The activation of signaling pathway via PRRs to certain PAMPs that leads to modulating the cellular and humoral immunity (Medzhitov and Janeway 2002).

Crustacean lectins are distinguished by the existence of carbohydrate recognition domain (CRD) with a unique aminoacid sequence motif, which involved mechanistic action of immune recognition, coagulation, phagocytic activity, melanization, agglutination, production of ROS complexes, and antimicrobial peptide (Cerenius et al. 2010b; Sritunyalucksana and Söderhäll 2000; Wang and Wang 2013). Due to this, it has ability to recognize non-self-recognition molecules like PRRs in crustacean immunity (Preetham et al. 2020; Sivakamavalli and Vaseeharan 2013). Among that, the structural and functional variety of the lectins in crustacea appears to demonstrate a vast range of immune actions relying on their sugar specificity.

Henceforth, the purification, molecular characterization, physiological relevance, and prompt upon infection in crustacean lectins have all been documented (Marques and Barracco 2000). Furthermore, the present chapter focused that molecular cloning and its functional interactions of crustacean lectins were explored.

8.2 Lectin as Carbohydrate Recognition Domain (CRD) Motifs

C-type lectins are Ca^{2+} dependent carbohydrate-binding lectins that mediate with a compact module termed as the "Carbohydrate Recognition Domain" (CRD). This domain interacts to a diversity of monosaccharides with specificity, but feeble calcium-dependent affinity (Drickamer 1993; Weis et al. 1998; Loris 2002). C-type lectin are initiate as building blocks in a diversity of multidomain proteins which intricated in the extracellular matrix, endocytosis, the primary immune system (Gabius 1997). To date, C-type lectins have been the most widely researched crustacean PRR family; nevertheless, how these lectins trigger immunological systems that affect intracellular signaling and regulatory processes is still unknown. Some of the lectin-CRD from numerous crustacean species are summarized in Table 8.1.

In the present report, the presence of ten different CRD lectins has been identified and has not been possible to identify a relationship between CRD motifs and the immunological activity in crustaceans. Further information is required to spot whether there is an association between CRD lectin specificity and immunological participation. Moreover, it is probable that the glycosylated ligands that bind the lectins could play a relevant role in regulating the immunological functions of crustaceans.

Table 8.1 Lectin-CRD from numerous species of crustacean

Crustacean species	Lectins	CRD motifs	Ref
Fenneropenaeus chinensis	Fc-Lec2[a] (40 kDa) FcLec3[a] (18, 18 kDa)	QPD, EPN, EPS	Zhang et al. (2009a) and Wang et al. (2009)
Litopenaeus vannamei	LvLec[a], LvCTL1[a] (15.8 / 16 kDa), LvLectin-2[a] (18.1 kDa), LvCTLD[b] (33/59 kDa), LvLT[a] (37.2 kDa)	EPN, QPD, QAP	Zhang et al. (2009b), Zhao et al. (2009), Wei et al. (2012), Junkunlo et al. (2012) and Ma et al. (2007)
Penaeus monodon	PmLec[a] (18 kDa), PmLT$^{N/D}$ (38 kDa)	QPD, EPN	Luo et al. (2006) and Ma et al. (2008)
Marsupenaeus japonicus	LdlrLec2[a] (32 kDa), LdlrLec1[a] (31 kDa)	QAP	Xu et al. (2014a, b)
Eriocheir sinensis	EsCTL (17 kDa), EsLec Ha (43 kDa)	EPQ, QPD	Wang et al. (2013) and Zhu et al. (2016)
Macrobrachium nipponense	MnCTLDcp1[a] (38 kDa)	EPL, WTD	Xiu et al. 2016;
Procambarus clarkii	Pc-Lec1$^{N/D}$ (23 kDa) PcLT[a] (20 kDa)	EPE, EPN	Zhang et al. (2011a) and Chen et al. (2013)

8.3 Lectin Carbohydrate Specificity

Several crustacean species have been intended to have a diverse spectrum of lectin specificity and their involvement in pathogen recognition (Table 8.2). *L. vannamei* species had the most lectins and their specificity reports. LvGal and LvCTLD lectins with galactose specificity are present in this organism (Hou et al. 2015; Junkunlo et al. 2012). In addition, both LvCTL1 and LvLec are selective for Man residues, whereas LvL detects GalNAc (Sun et al. 2007; Zhang et al. 2009b; Zhao et al. 2009).

M. japonicus has a diversity of lectins with assorted carbohydrate specificities. LdlrLec1 as well as LdlrLec2 are calcium-dependent lectins that identify VP28 (WSSV envelope protein) (Xu et al. 2014a, b). The lectins MjLecA, MjLecB, and Mj LecC are specific for GalNAc, GlcNAc, and Man, respectively (Song et al.

Table 8.2 Specificity of lectin crustaceans from multi-species

Lectin from Crustacean species	Lectin specificity
Balanus rostratus	Gal, GalN, GalNAc, Neu5Ac
Cancer antennarius	Neu4Ac, Neu5,9Ac2, Neu5,7,8,9Ac4
Fenneropenaeus chinensis	Gal
Homarus americanus	GalNAc, Neu5Ac
Jasus novaehollandiae	PSM, asialo-PSM, Fetuin, D-ribose, D-arabinose, D-galactose
J. verreauxi	BSM; Fetuin; NeuAc; GlcNAc
Scylla serrata	NeuGc; Colomic acid; thyroglobulin; BSM; Fetuin
C. japonicus	α-Galactosyl; α-glucosyl
C. antennarius	NeuAc; BSM; ESM
Liocarcinus depurator	Neu5,9Ac, Neu,5,7,8,9Ac4
Litopenaeus schmitti	GlcNAc, GalNAc, Neu5Ac, Fetuin, ManAc
Litopenaeus setiferus	GlcNAc, GalNAc, Neu5Ac, MSB, Fetuin
Litopenaeus vannamei	GlcNAc, GalNAc, Neu5Ac
Macrobrachium rosenbergii	GlcNAc, GalNAc, Fetuin, Neu5Ac; Neu5, 9Ac2
Megabalanus rosa	Man-type, gal-type, Neu5Ac
Penaeus californiensis	GlcNAc, GalNAc, ManAc, Fetuin, MSB
Penaeus indicus	GlcNAc, GalNAc, Neu5Ac, MSB, MEP, Fetuin
P. longirostris	GalNAc, NeuAc, Fetuin, LSP, Gal
M. japonicus (P. japonicus)	GalNAc, Neu5Ac, Ribose, MSB, MEP, Fetuin
Penaeus monodon	GlcNAc, GalNAc, Neu5Ac, Fetuin, LSP
Paratelphusa jacquemontii	Neu5Ac, Neu5,9Ac2, Neu5,8,9Ac3
Potamon potamios	α-Gal
P. leniusculus	PSM; BSM; Fetuin; LPS; GlcNAc; α-1-rhamnose
Austropotamobius pallipes	Fetuin
Astacus astacus	Fetuin; D-xylose
Astacus leptodactylus	Fetuin
Procambarus clarkii	Fetuin; GalNAc; GlcNAc, Maltose
Squilla mantis, lect anti-H	L-Fucose
Squilla mantis, lect anti-A	GalNAc

2010). *M. japonicus* lectins (MjLTL1 and MjHeCL) recognize lipopolysaccharides in which *M. japonicus* galectin (MjGal) has a strong specificity for lipoteichoic acid, the most abundant molecule presents in $Gram^+$ bacteria (Wang et al. 2014a; Xu et al. 2014a, b). The lectins LdlrLec1 as well as LdlrLec2 could also interact with a VP28 (WSSV) protein (Shi et al. 2014). The lectins from *F. chinensis* are calcium-dependent C-type lectins in which Fc-Lec2, FcLec3, and FcLec4, mostly identify peptidoglycans. (Wang et al. 2009, 2014b; Zhang et al. 2009a). *E. sinensis* crab lectins (EsCTL, EsLecB, EsLecH, and EsERGIC-53) specially recognize LPS. EsVIP36 lectin has specific recognition of Man and ManNAc (Huang et al. 2014; Fang et al. 2016; Wang et al. 2013; Zhu et al. 2016).

The shrimp *L. vannamei* possesses three lectins which are specific for Gal (LvCTLD, LvCTL1, and LvGal), while LvLec and LvL lectins can bind with $Gram^-$ bacteria (Sun et al. 2007; Zhao et al. 2009; Junkunlo et al. 2012; Zhang et al. 2009b; Hou et al. 2015). The lectin from *L. setiferus* (LsL) and *L. schmitti* recognize NeuAc as well assialylated O-glycosylated proteins (Alpuche et al. 2005, 2009; Cominetti et al. 2002).

In the *Penaeus* sp., some lectins have been found in different species. *P. monodon* possesses a Gal-specific lectin (PmLec) and recognizes different types of LPS (Luo et al. 2006). Interestingly, *P. californiensis* lectin (BSH-I), *P. indicus* lectin (Lectin A), *P. japonicus* lectin (PjLec) could bind the same kind of carbohydrates, preferably NeuAc and N-acetylated sugars (Maheswari et al. 1997; Vargas-Albores et al. 1993; Yang et al. 2007).

In *Macrobrachium* sp., some lectins have been observed. *M. rosenbergii* was reported with specificity for N-acetylated sugars. In *M. nipponense* prawn, three lectins (MnCTLDcp1, MnCTLDcp2, and MnCTLDcp3) were identified that could bind $Gram^-$ and $Gram^+$ bacteria (Vazquez et al. 1996; Xiu et al. 2015). Among other crustaceans, *F. merguiensis* (FmL) lectin that has specificity for ManNAc, GlcNAc, GalNAc, and NeuAc (Rittidach et al. 2007). Man, and Mannan are molecules found in fungus species, and *S. serrata* lectin (HA) recognizes them (Jayaraj et al. 2010).

It is important to note that many lectins have been identified by molecular techniques and that their expression has been evaluated in different tissues. Though, their specificity has still to be determined. Additionally, it is not clear if the specificity of the lectins is related to the activation of a specific immune mechanism in crustaceans.

8.4 Structural Aspects of Crustacean Lectin

Crustacean lectin is classified based on their structural homology (Fig. 8.1). There are C-type lectins, mannose-binding lectins (P-type), and galectins lectins (S-type). In each family, it has comparable sequences and structural properties (Lis and Sharon 1998; Anderson et al. 2008). Nowadays, M-type, L-type, chitinase-like, and F-type lectins have recently been found. There is currently no broadly accepted

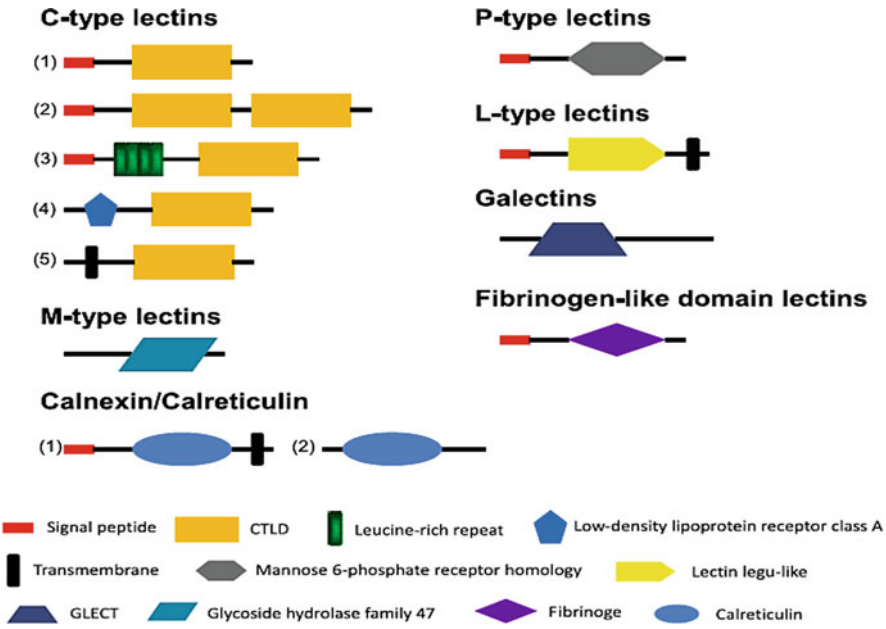

Fig. 8.1 Structural homology of different types of lectins. (Source: Wang XW, Wang JX. Diversity and multiple functions of lectins in shrimp immunity. Developmental & Comparative Immunology. 2013 Jan 1;39(1–2):27–38. (Copyright obtained from journal of "Developmental and Comparative Immunology"))

classification of lectins. In this chapter, some conventional animal lectins families are described.

Lectins have a vast range of amino acid sequences which found in bacteria, viruses, animals, and plants. They have a variety of activities, structures, tissue localizations, and carbohydrate specificity. Although animal lectins do not have homologous primary structures but they have similar binding to carbohydrates (De Schutter and Van Damme 2015). In crustacean lectin families and their localization were described in Table 8.3.

8.4.1 C-Type Lectins

Endocytic receptors known as C-type lectins are primarily expressed by macrophages, DCs, and certain endothelial cells. It requires Ca^{2+} and unique sequence motif of invariable (14) and vastly bounded amino acid (18) residues. Further, it can induce cytokine production and recognize diverse bacterial pathogens in antibacterial immunity. Also, they are intricated in homeostasis and immunomodulatory (Gordon 2002). Many C-type lectins play primary roles in immunity. There are reports related to C-type lectin (CTL) in different crustacean species have been

Table 8.3 Localization of crustacean lectin families

Crustacean lectin family	Typical ligands	Localization
Calnexin & Calreticulin	Glc1Man9	ER
M-type lectins	Man8	ER
L-type lectins	Various	ER, Golgi
P-type lectins	Man6-phosphate	Secretory pathway
C-type lectins	Mannosides, galactosides, sialic acids and others	Membrane bound, extracellular
S-type lectins (galectins)	β-Galactosides	Cytosol, extracellular
I-type lectins (siglecs)	Sialic acid	Membrane bound
R-type lectins	Various	Golgi, membrane bound
F-box lectins	GlcNAc2 of N-glycans	Cytoplasma
Fibrinogen-type lectin	GlcNAc, GalNAc	Membrane bound, extracellular
Chi-lectins	Chito-oligosaccharides	Extracellular
F-type lectins	Fucose terminating oligosaccharides	Extracellular
Intelectins	Galactose, galactofuranose, pentoses	Membrane bound, extracellular
Annexins	Glycosaminoglycans, heparin and heparin sulfate	Membrane bound

identified such as. *M. rosenbergii* (Huang et al. 2016a), *E. sinensis crab* (Zhu et al. 2016), *M. nipponense* (Huang et al. 2019), *M. japonicus* shrimp (Wang et al. 2014a, b), *P. clarkii* crayfish (Zhang et al. 2016), *P. monodon* (Luo et al. 2006), and *L. vannamei* shrimp (Zhang et al. 2019a).

8.4.2 Fibrinogen-Related Proteins (FREPs)

Fibrinogen-related proteins (FREPs) contain a fibrinogen-related domain (FReD) related to the immune response, which is responsible for carbohydrate binding. Ficolins are proteins that belong to the FREPs family; crustaceans have a considerable number of FREPs related to bacterial responses. There are some reports related to FREPs are *M. nipponense* ficolins: MnFico1, MnFico2, and MnFico3 (Li et al. 2017; Qin et al. 2020). *M. japonicus* FREP (MjFREP2) (Sun et al. 2014), *F. merguiensis* FmLFd (Senghoi et al. 2019), *P. leniusculus* and *P. clarkii* crayfishes, and *L. vannamei* (Wu et al. 2011; Chen et al. 2016; Dai et al. 2017; Tian et al. 2018a), Three ficolins of *M. rosenbergii*, MrFico1, MrFico2, and MrFREP (Zhang et al. 2014; Han et al. 2018).

8.4.3 L-Type Lectins

L-type lectins (LTL) have a CRD that resembles that of leguminous lectins. LTLs are diversely distributed and exert different functions in the cell; these lectins can interact with N-glycans from glycoproteins involved in cellular trafficking. Several L-type lectins have been identified as being involved in bacterial clearance (Sharon and Lis 2004; Kamiya et al. 2008). Reports in LTL species are *M. japonicus* shrimpMjLTL1 (Xu et al. 2020), *L. vanammei* shrimp (LvLTLC1) (Tian et al. 2018b), *E. sinensis* crab (EsERGIC-53 and EsVIP36) (Huang et al. 2014), and *M. rosenbergii* MrVIP36 (Huang et al. 2018). Furthermore, PcL-lectin was able to enhance the multiplication of WSSV, suggesting its participation in the viral replication process (Dai et al. 2016).

8.4.4 Mannose-Binding Lectin (MBL)

Mannose-binding lectin (MBL) is a C-type lectin that plays a crucial role in the first line of host defense. In vertebrates, this protein activates the complement system, induces phagocytosis, and assembles the membrane attack complex (Eddie Ip et al. 2009). However, although some molecules show homology with the vertebrate complement system, this system has not been fully identified in crustaceans. Nevertheless, the proPO system might carry out some of the functions of the complement system (Cerenius et al. 2010a). A recent study performed in *C. quadricarinatus* crayfish identified an MBL-like protein in hemocytes, suggesting that this MBL could be related to a protective effect, preventing damage from its own immune system and found in *P. trituberculatus* crab and *M. rosenbergii* crayfish could inhibit the growth of several pathogens (Arockiaraj et al. 2015; Zhang et al. 2019b; Pereyra et al. 2020).

8.4.5 Galectins

Galectins are a family of β-galactoside-binding proteins (previously, S-type lectins) that can recognize endogenous self-glycans and mediate several processes, including cell differentiation, tissue organization, and immune homeostasis (Vasta 2009). In invertebrates, galectins have unique structural features and domain organizations, showing the same or different CRD subunits; currently, it is clear that galectins also identify glycans on pathogenic microorganisms (Vasta and Wang 2020). Galectins reported in crustaceans are related to the cellular immune response such as *M. japonicus* MjGal (Shi et al. 2014), *L. vanammei* LvGal (Hou et al. 2015), and *E. sinensis* EsGal (Wang et al. 2016).

8.4.6 *Miscellaneous Lectin Families*

There are reports on lectins that belong to different family groups. In *M. nipponense*, an M-type lectin has been identified (MnMTL1) that is overexpressed after infection with *A. hydrophila* and *A. veronii*, suggesting that this lectin plays a role in the immune response (Xiu et al. 2015). Two proteins with homology to calnexin (Cnx) and calreticulin (Crt) have been identified in *E. sinensis* crab, EsCnx and EsCrt, which are located in the endoplasmic reticulum with LPS and PGN carbohydrate-binding activity, respectably. These molecules are involved in the clearance process of *V. parahaemolyticus* in an in vivo model (Huang et al. 2016b).

8.5 Molecular Cloning of Crustacean Lectin

In crustacean lectin, C-type lectins are the most studied crustacean PRR family to date; however, how these lectins trigger the immunological systems that regulate intracellular signaling pathways and regulatory processes is still unclear. In vertebrates, the C-type lectin family is currently classified into 17 groups and its relying on the architecture of the C-type lectin domain-containing proteins. Although various papers on C-type lectins in invertebrates have been published, there is little evidence available for the classification of the C-type lectin family at the gene and protein levels.

In crustaceans, many C-type lectins have been identified, and most of these lectins display anti-virus and anti-bacteria activities (Wang et al. 2013). In account that, the sequence information of many crustacean CTLs have been reported, including some sequences submitted online directly to the database. The available sequences were listed in Table 8.4.

C-type lectins have been found to be highly conserved in vertebrates but are considerably more diverse among invertebrates. Recently, a limited number of C-type lectin cDNAs have been reported from crustacean species and all are involved in the shrimp challenges against bacteria or viruses.

In molecular cloning, the cDNA sequence was reported from *P. trituberculatus* C-type lectin (PtCTL4 MF946551) with 654 bp (Zhang et al. 2018). In hemocytes of *P. fucata* (F-type lectin JX103557) cDNA was cloned with 588 bp coding for196 aa (Anju et al. 2013). Likewise, Chinese shrimp *F. chinensis* C-type lectin (Fclectin AY871270) was cloned with 1482 bp encoding 287 aa (Liu et al. 2007). The hepatopancreas of banana shrimp *F. merguiensis* C-type lectin (FmLCACR56805) was cloned with 1118 bp encoding 333 aa (Rattanaporn and Utarabhand 2011). Furthermore, the stomach of the banana shrimp *F. merguiensis* C-type lectin (FmLC2 KC894154) was cloned with 1098 bp & 245 aa (Runsaeng et al. 2015). In Chinese mitten crab, *E. sinensis* (Es-Lectin ADB10837) was cloned and characterized with 651 bp encoding 160 aa (Zhang et al. 2011b). Also, hepatopancreatic cDNA of *E. sinensis* CTL (EsLec DJX129177) was identified with 686 bp encodes

Table 8.4 The sequencing information of crustacean lectin

Species	Name	GeneBank No.	CTLD	Length of CTLD	Ca^{2+}-binding site 2	Special feature
F. chinensis	FcLec1 (Fc-hsL)	ABA54612	1	131	EPN, WYD	
	FcLec2	ACJ06428	2	134, 134	QPD, MND EPN, FRD	
	FcLec3	ACJ06431	1	130	EPS, FAD	
	FcLec4	ACJ06432	1	174	QPD, WHD	
	FcLec5	ACJ06429	2	134, 134	QPD, LND EPQ, FRD	
	FcLec6	ACJ06429	1	144	QPD, WHD	
	Fclectin	AAX63905	2	123, 123	QPD, VND QPD, VND	
P. monodon	PmLT	ABI97373	2	134, 134	QPD, MND EPN, FRD	
	PmLec	AAZ29608	1	123	QPD, LND	
	PMAV	AAQ75589	1	134	QPD, IHD	
L. vannamei	LvLT	ABI97374	2	134, 136	QPD, MND EPD, FRD	
	LvLec	ABU62825	1	131	EPN, WYD	
	LvCTL	ADU25463	2	134, 136	QPD, MAD –, F(L)RD	Mutant of LvLT
	LvCTL1	DQ858900	1	131	EPN, WYD	
	LvCTLD	AEH05998	1	159	QAP, –	LDLa
	LvLec1	ADW08727	1	137	QPD, –	
	LvLec2	ADW08726	1	134	QPN, –	
M. japonicus	MjLecA	ADG85666	1	–	EPS, FND	
	MjLecB	ADG85668	1	133	QPT, FND	
	MjLecC	ADG85667	1	135	QPN, FHS	
	MjLecD	ADG85659	1	132	QPD, LTD	
	MjLecE	ADG85658	1	131	EPN, WYD	
	MjLec1	JQ804928	1		–, FDD	LRR
	MjLec2	JQ804929	1		–, –	LDLa
	MjLec3	JQ804930	1		–,–	TM
F. merguiensis	FmLC	ACR56805	2	134, 134	QPD, MND EPN, FRD	
Penaeus semisulcatus	PsCTL	ABI97372	2	134, 134	QPD, EPN	
Penaeus stylirostris	PstCTL1		1	139	EPK, FDD	No signal peptide

– Undetectable. LDLa, low density lipoprotein receptor class A; LRR, leucine rich repeats; TM, transmembrane.

Source: Wang XW, Wang JX. Diversity and multiple functions of lectins in shrimp immunity. Developmental & Comparative Immunology. 2013 Jan 1;39(1–2):27–38. (Copyright obtained from journal of "Developmental and Comparative Immunology")

155 aa (Guo et al. 2013). In sea cucumber *A. japonicus*, a C-type lectin (AJCTL JN133520) cDNA with 710 bp encodes 205 aa (Han et al. 2012). A novel isoform cDNA (ALFSp2 HM345950) of anti-lipopolysaccharide factors (ALFs) was cloned from the mud crab, *S. paramamosain* with 348 bp encodes 115 aa residues were reported (Imjongjirak et al. 2011). The putative C-type lectin-like domain from gills of *L. vannamei* (CTLD MGID1052359) with 198 bp downregulated in YHV-infected shrimp (Junkunlo et al. 2012). Thenceforth, PmLec cDNA of *P. monodon* with 546-bp comprising 182 aa (Luo et al. 2006). The *L. vannamei* lectin cDNA (LvLTDQ871245) was cloned with 1035 bp encoding 345 aa (Ma et al. 2007). Furthermore, giant freshwater prawn *M. rosenbergii* cDNA of 4 CTL (JQ349147) genes were cloned (MrLec1, MrLec2, MrLec3, and MrLec4). All of these 4 lectin cDNAs encode with two CRDs with 1378 bp encodes 322 aa (Ren et al. 2012). In *M. japonicus,*three lectins (MjLecA, MjLecB, and MjLecC) were cloned with WSSV envelope proteins (Song et al. 2010). FmLC1 (KC894157 and AGS42196) C-type lectin was cloned from hepatopancreas of banana shrimp *F. merguiensis* with 706 bp encodes 158 aa (Thepnarong et al. 2015). Ec-CTLFJ805451 was cloned from grouper *E. coioides* with 840 bp that encodes 216 aa(Wei et al. 2010). M-type lectin and L-Type lectin cDNA (MnMTL1 &

MnLTL1-KP123625) from river prawn *M. nipponense* with 2064 bp encodes 586 aa (Xiu et al. 2015). C-type lectin gene (LvLecEF583939) cDNA was cloned from hemocytes of *L. vannamei* with 618 bp (Zhang et al. 2009a, b). CTL, SpCTL5, was identified from the hepatopancreas of the mud crab *S. paramamosain* with 762 bp encoding 253 aa (Zhang et al. 2019c). CTL cDNA was cloned from *M. japonicus* (MjCTL MN203536) with 513 bp encoding 170 aa and its tissue distribution and expression patterns over time in response to white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* were reported (Zheng et al. 2020).

8.6 Computational Analysis of Crustacean Lectins

In numerous reports, the lectins structures and their sugars complexes have been studied via X-ray crystallography and NMR spectroscopy. These are based on new theoretical techniques as well as credible experimental data (Neumann et al. 2004), but there was no literature for crustacean lectins in computational analysis. The computational data of lectin from other sources (plant and bacteria) were carried out on Linux workstation using, ChemBio3D Ultra 11.0, AutoDock 4.2, Discovery Studio 4.0, Schrödinger and PyMol software. In that, protein and ligand structure optimization, molecular docking, and molecular electrostatic potential (MEP) mapping were described. Besides, the homology modeling was determined via SWISS-MODEL server. SAVES web server was implemented for inspecting stereo-chemical quality of the modeled structure. Protein–protein docking was done with a web-based server ZDOCK. PRODIGY and PP Check web servers were used for interaction analyses and the COCOMAPS web server was utilized for studying the properties of the interacting two proteins complexes (Vinod et al. 2021).

8.7 Functional Interactions of Crustacean Lectin

In crustacean, the functional heterogeneity of the lectins relying on its sugar specificity seems to be varied in innate immune responses. This chapter attempts to summarize the reported role of immunological functions in crustacean lectins (Fig. 8.2).

In immune actions have been determined which initiates as phagocytosis, induction of the prophenoloxidase activating system, and activation of respiratory burst. It was also found to play important roles in antiviral immunity. Furthermore, basic agglutination is an effective way to resist bacterial pathogens (Wang et al. 2013; Sánchez-Salgado et al. 2017). The functional interaction of crustacean lectin from different species was described in Table 8.5.

In several organisms, lectin's cellular receptors have been notorious in hemocytes. This information suggests that these cells participate in the activation and

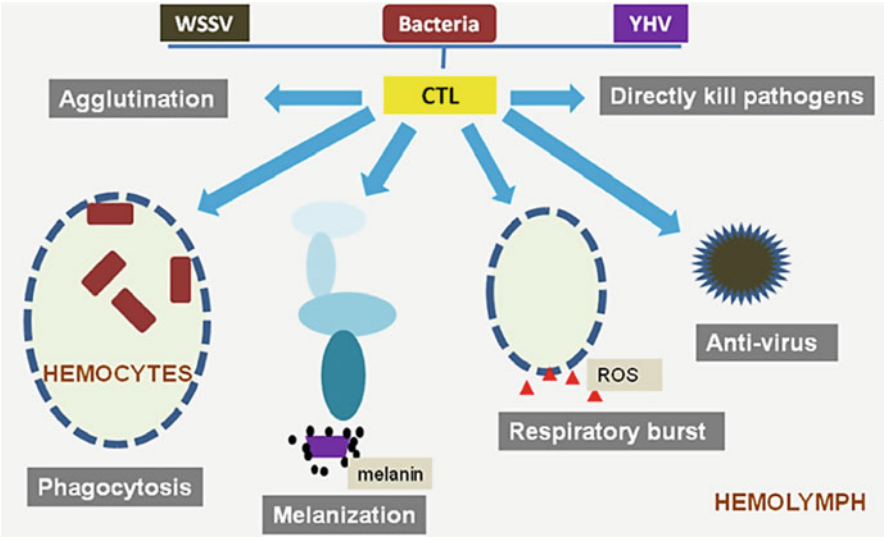

Fig. 8.2 Role of immunological functions in crustacean lectins. (Source: Wang XW, Wang JX. Diversity and multiple functions of lectins in shrimp immunity. Developmental & Comparative Immunology. 2013 Jan 1;39(1–2):27–38. (Copyright obtained from journal of "Developmental and Comparative Immunology"))

regulation of the immune mechanism. However, more tissues could present lectin receptors and participate in the modulation of immunological mechanisms. For instance, the hepatopancreas seems to express receptors for lectins that modulate immune mechanisms (Sánchez-Salgado et al. 2017). The crustacean lectins appear to have diversified with common function of PRR.

8.8 Conclusion

To date, many lectins have been identified in crustaceans. Most of the published reports identified and sequenced lectins at the molecular and transcriptional levels. However, some publications have reported on the characterization of lectins on the protein level, thus more information on the lectin carbohydrate interaction is needed to elucidate their contribution in the immune system over sugar recognitions. To the best of the author's knowledge, among crustaceans, there are only a few reports on the lectin-receptor interaction and how this recognition triggers the immune response. Although several lectins have been identified, the regulation mechanisms of their expression after an immunological challenge and their relationship to a specific group of pathogens remain unknown; more information are required to clarify this mechanism. It is important to determine how lectins and their specificity for carbohydrates participate in the regulation of the immune response in the

Table 8.5 Functional interactions of crustacean lectin from different species

Functional interactions activities	Lectin species	Tissue expression
Agglutination activity	*Fenneropenaeus chinensis*:FC-L[a] (40, 34 kDa); Fc-Lec2[a] (40 kDa); FcLec3[a] (18, 18 kDa)	Hemolymph and Hepatopancreas
	Fenneropenaeus merguiensis:FmLa (32, 30 kDa)	Hemolymph
	Litopenaeus schmitti: *L. schmitti* lectinb (31, 34 kDa)	Hemolymph
	Litopenaeus vannamei: LvLeca *Litopenaeus vannamei*: LvLa (32, 38 kDa)	Brain Hemolymph
	Penaeus californiensis: BSH-Ia (41 kDa)	Hemolymph
	Penaeus indicus: Lectin A[b]	Hemolymph
	Penaeus japonicus: PjLecb (37 kDa)	Hemolymph
	Macrobrachium nipponense: MnCTLDcp1[a] (38 kDa); MnCTLDcp2[a] (38 kDa); MnCTLDcp3[a] (38 kDa)	Heart and Hepatopancreas
	Eriocheir sinensis: EsERGIC-53[a] (56 kDa); EsVIP36[a] (36 kDa)	Hepatopancreas
	Scylla serrata: HA[a]	Hemolymph
Antiviral activity	*Marsupenaeus japonicus*: LdlrLec1a (31 kDa); LdlrLec2[a] (32 kDa); MjLecA[b] (19 kDa); MjLecB[b] (20 kDa); MjLecC[b] (17 kDa)	Hemocytes and Hepatopancreas
	Litopenaeus vannamei: Lv lectin – 2[a] (18.1 kDa); LvCTL1[a] (15.8/16 kDa; LvLT[a] (37.2 kDa)	Hepatopancreas
Phagocytosis	*Litopenaeus setiferus*: LsLa (80, 52 kDa)	Hemolymph
	Litopenaeus vannamei: LvGal N/D (60 kDa)	Gills
	Marsupenaeus japonicus: MjGala (33.5 kDa); MjLTL1a (38 kDa)	Hemocytes and Hepatopancreas
	Penaeus monodon: PmLeca (18 kDa)	Hemolymph
	Fenneropenaeus chinensis: Fc Lec 4[N/D]	Stomach
	Paratelphusa jacquemontii: *P. Jacquemontii* Lectin[N/D] (320 kDa)	Hemolymph
	Procambarusclarkii: PcLT[a] (20 kDa)	Hepatopancreas
	Macrobrachium rosenbergii: MrL[a] (20 kDa)	Hemolymph
	Cherax quadricarinatus: CqL[a](290 kDa)	Hemolymph
	Procambarus clarkia: P*c Lec 3* [N/D]	Hemocytes
Encapsulation	*Penaeus monodon*: PmLT[N/D] (38 kDa)	Hepatopancreas
	Eriocheir sinensis: EsCTL (17 kDa); EsLecB[b]	Hepatopancreas and Hemocytes
	Procambarus clarkii: Pc-Lec1[N/D] (23 kDa)	Gills
	Litopenaeus vannamei: LvCTLD[b] (33/59 kDa)	Gills
proPO	*Penaeus semisulcatus*: Ps-Lec[N/D] (118 kDa)	Hemolymph
	Procambarus clarkii: PcLec2[a] (16 kDa)	Hepatopancreas
Antimicrobial peptide	*Marsupenaeus japonicus*: MjHeCL[a]	Hemocytes
	Eriocheir sinensis: EsLecH[a] (43 kDa)	Hemocytes

crustaceans, and this information will serve as a powerful tool for the global aquaculture industry.

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Conflict of Interest The authors declare no conflict of interest.

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Part III
Molecular Mechanism of Lectin Activity

Chapter 9
Fish Lectins in Host–Pathogen Interaction

Nivya Mariam Paul, K. K. Dayamrita, and Nayomi John

Abstract Host–pathogen interactions are complex, governed by numerous factors such as host's age, environment, behavior, immune system, and characteristics of the pathogen. The outcome of such a relationship depends on the resistance and susceptibility of the immune reactions of host. In fishes, there are two type immune responses, adaptive and innate. One of the key components of innate immune response is lectins. Lectins attach to the carbohydrate component of the glycoproteins or lipids, proteoglycans, and are basically proteins. They not only mediate protein–carbohydrate interactions for the identification of infectious agents but also involved in biological activities of immune system like immobilization, agglutination, opsonization, and inactivation of pathogens. Studies on identification and characterization of lectins in fish are essential for prophylaxis, tracking, and treatment of diseases.

Keywords Host–pathogen · Immune system · Fish lectins

Abbreviations

Bf	Factor B
Ca^{2+}	Calcium
$CD4^{+}$	Cluster of differentiation 4
CTL	Cytotoxic T lymphocyte
CRD	Carbohydrate-recognition domain
E. coli	*Escherichia coli*
EST	Espressed sequence tag
FTLs	Ferritin light chain
Gb3	Globotriasylceramide
IHNV	Hematopoietic necrosis virus
ISKNV	Infectious spleen and kidney necrosis virus

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LPSs	Lipopolysaccharides
MASPs	MBL-associated serine proteases
MBL	Monoclonal B-cell lymphocytes

9.1 Introduction

Unlike invertebrates, vertebrates contain an internal skeleton of bone or cartilage. The jawless fish (hagfish, lamprey), cartilaginous fish (rays, sharks), bony fish (trout, carp), amphibians, birds, reptiles, and mammals are included in Vertebrata subphylum. Fishes are the oldest set of animals with immune systems exhibiting resemblances with the immune systems of birds and mammals (Van Muiswinkel and Vervoorn-Van Der Wal 2006). The immune systems of an organism defend against diseases by removing an infectious agent and repressing the rise of tumors. It maintains homeostasis during the growth and development of the organism following tissue damage or inflammatory reactions (Magnadottir 2010). Immune systems are of two types: adaptive and inborn. These systems react spontaneously (minutes to hours) without depending on the specific epitopes on the pathogens. It is also temperature independent. On the other hand, the acquired immune system usually takes weeks or months to act against pathogens. In acquired immunity, the immune cells specifically bind to the pathogens, and they produce memory cells (Van Muiswinkel and Vervoorn-Van Der Wal 2006).

Proteins with CRD or proteins with domains that can recognize the carbohydrate are known as lectins. Monomeric or membrane-bound proteins that are unable to cause cell agglutination are also considered as lectins. Based on biological function and sequence data, in animals, the crucial types of lectins are C and S. C-type (extracellular and membrane proteins) lectins have Ca^{2+} binding CRD, and the domains are rich in S–S bond. On the other hand, S-type (both extracellular and intracellular) lectins have no S–S bonds and distinguish galactose (Drickamer and Taylor 1993).

Lectins are present in plants, viruses, protists, bacteria, fungi, and animals (Mirelman 1986). The extensive diversity of microbial pathogens utilizes lectins that recognize glycans present in the host cell surface and colonizes on the cell surface (Mandlik et al. 2008). Specific adhesins like fimbriae and pili on bacterial surfaces are known to mediate interactions among bacterial pathogens and the precise cell of the host. The virulence and colonization component of numerous pathogens (Frederick and Petri Jr 2005; Von Itzstein et al. 2008) and the key identification factors that permit either favorable interaction or immune reaction opposing pathogens are humoral and membrane-related lectins. Furthermore, host lectins facilitate downstream effector functions such as immobilization, agglutination, inactivation, and phagocytosis (Vasta et al. 2004a) and control adaptive immunity. They modulate dendritic, B-cell, and T-cell activation cell differentiation, and embryonic development (Rabinovich and Toscano 2009).

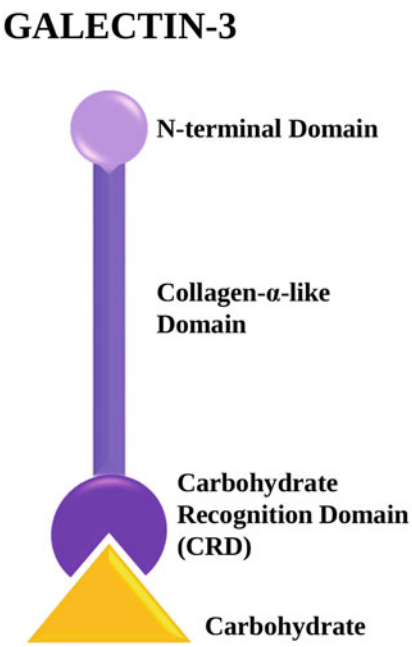

Fig. 9.1 Galectin-3 binding to carbohydrate through carbohydrate recognition domain (CRD).

Lectins in animals are distinguished by the presence of a CRD which are bound to the oligomers of peptides by either a non-covalent or a covalent bond (Taylor and Drickamer 2011). Amino acid conserved sequences are seen within the CRD, and they have distinct domains and features like reducing habitat for ligand binding, demand of divalent cations, etc. Such properties have led to the classification of lectins into several families. Lectin subunit (membrane associated or soluble) can exhibit an array of biological activities due to its structural diversity. A type of S-lectin, Galectin-3 binding to carbohydrate through CRD is depicted in Fig. 9.1. CRD is connected to different functional areas of lectin (e.g., F and C) or a subunit of lectin peptide can comprise numerous CRDs which are jointly arranged (Zelensky and Gready 2005; Odom and Vasta 2006).

9.2 Immune Relevant Lectins in Fish

Several lectins have been described from fish, and these have been characterized based on agglutination activity and carbohydrate specificity. Some of the lectins relevant to the immune systems of fish have been shown in Table 9.1.

Table 9.1 Immune relevant lectins

Categories	Specificity	Organisms	Features	Biological activity	References
S-type lectins (galectins)	Galactosides	Catfish (*Ictalurus punctatus*), trout (*Oncorhynchus mykiss*)	Ca^{2+}-independent activity S-type sequence motif	Inflammatory responses, apoptosis, tumor metastasis	Cooper (2002), Fukumori et al. (2007)
C-type	Man, Gal, Fuc	Grass carp (*Ctenopharyngodon idellus*) Japanese flounder (*Paralichthys olivaceus*)	Ca^{2+}-dependent activity C-type sequence motif	Innate immunity, promote phagocytosis, activation of complement (MBL)	da Silva Lino et al. (2013), Kerrigan and Brown (2009)
Calnexin	Glc_1 Man_9	Rainbow trout (*O. mykiss*) Cat fish (*I. punctatus*)	Intracellular lectin Calnexin sequence motif	Stress-induced apoptosis Folding mechanism and misfolded protein retention	da Silva Lino et al. (2013), Williams (2006), Takizawa et al. (2004)
I-type	Variable	Zebrafish (*D. rerio*) Fugu (*T. rubripes*)	Affinity for sialic acid Ig-like domains	Cell–cell interactions, cell routing	Lehmann et al. (2004), Varki and Angata (2006)
F-type	L-fucose	Nile tilapia (*O. niloticus*) Striped bass (*Morone saxatilis*)	Ca^{2+}-independent activity Non glycosylated F-type sequence motif	Molecular recognition	Salerno et al. (2009), da Silva Lino et al. (2013)

Pentraxins	Pc/galactosides	Atlantic cod (*Gadus morhua*), pangasius (*Pangasianodon hypophthalmus*)	Ca^{2+}-dependent activity Multimeric binding motif Acute phase serum protein	Foreign cell recognition, endocytosis, complement activation	Kilpatrick (2002), Gisladottir et al. (2009), Giang et al. (2010), Magnadóttir et al. (2010)
Intelectins (X-type)	Gal, pentoses, galactofuranose	Grass carp (*Ctenopharyngodon idella*)	Ca^{2+}-dependent activity Intelectin sequence motif	Pathogen surveillance, agglutination	Vasta et al. (2011), Chen et al. (2020)

9.3 Host–Pathogen Interaction in Fish

Host–pathogen interactions are complex relationships that can favor either the pathogen or the host based on several factors. While the pathogen attempts to establish an infection in the host, the host uses defense mechanisms to resist the infection. Factors such as host susceptibility and resistance will decide whether or not the infection becomes established. Factors that influence infectivity and susceptibility are the mode of entry of the pathogen, nutritional requirements, ability to dodge host defense mechanisms, thrive in a site of an inadequate immune response, and mimic host's protein composition. The host's environment such as water temperature and crowding, feeding habits, locality in the column of water, age, behavior, immunological, and physiological conditions are some other factors. Certain factors affecting fish diseases are depicted in Fig. 9.2

Since fish health status is conditioned to or reliant on the environment, they are exposed to extensive diversity of opportunistic and pathogenic microorganisms. They may inhabit the skin or the internal body parts of fish. Hence, the formation of a protecting microflora is a crucial component in maintaining health and eliminating invaders. This is achieved by elimination processes that enable the growth and development of the immune systems (Gómez and Balcázar 2008).

An equilibrium exists between the defense response of the host and the pathogen's mechanisms of infection. The rate of mortality usually increases when the host–pathogen interaction is recent or when the defense mechanisms are compromised.

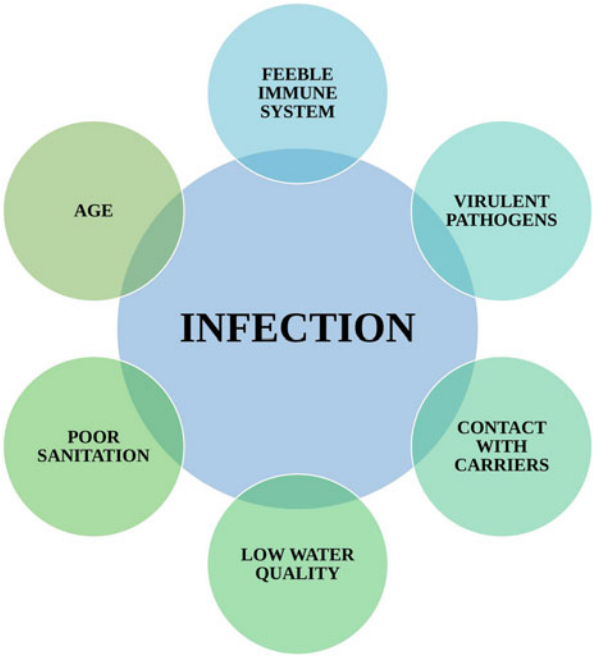

Fig. 9.2 Factors affecting fish infections

There will be developmental stress on the infectious agent to induce minimum hurt or avoid and cope with host defense mechanisms. A pathogen may survive due to its rapid proliferation or via host destruction. (e.g., infection of turbot by *Enteromyxum scophthalmi)* (Sitjà-Bobadilla et al. 2006).

One of the factors determining the antigenicity of pathogens may be the route that will evoke various responses in the host (Quillet et al., 2007). Innate immunity is the primary protection mechanism of fish, and most pathogens employ several means to escape or delay this. These mechanisms include infection of areas where an immune reaction is usually repressed, such as the central nervous system, gonads and the eyes, or antigen-based approaches like mimicking, rapid variations or masking of antigen, avoiding humoral defense parameters. Metazoan parasites escape the host's complement system attack by obstructing the complement cascade (Schroeder et al. 2009; Magnadottir 2010).

9.3.1 Host Lectins

Host lectins provide protection against pathogen invasion via inhibiting the reproduction and growth of pathogens or by promoting the host immune reaction. They bind to surface polysaccharides, peptidoglycans, teichuronic acids, or lipopolysaccharides (LPSs) of a pathogen, interrupt functions of the cell membrane, and thus prevent pathogens (Iordache et al. 2015). Membrane-bound lectins identify the extracellular glycans in microorganisms and convey signals to activate biological responses. Host lectins also help pathogen transmission and spread. The lectin DC-SIGN, on dendritic cells, bind with the glycans in virus surface and undergo endocytosis (Chatterjee et al. 2012), or the viral epitopes bind to dendritic cells, move to lymph nodes, and present to $CD4^+$ which trigger adaptive immune responses to stop the infection (Lin et al. 2020).

9.3.2 Pathogen Lectins

The lectins in infectious agents sense the host and promote infection by suppressing the host immunity. The extracellular glycans, glycolipid, or residual glycoprotein on host and the lectins on the antigen surface are crucial factors that affect this process. They can bind to intracellular sugars disrupting the sucrose signal and suppressing the immune response (Lin et al. 2020).

9.4 Lectins in Host–Pathogen Interaction

The most sophisticated internal defense system can be regarded as the antibody-mediated immune responses as they have affinity maturation and memory. The acute phase reactions and identification of a broad range of microorganisms enable the host to eradicate an infectious agent before activating the complete immunological responses (Vasta et al. 2011).

Lectins are endowed with recognition and effector functions. Along with the precise binding, agglutinating, and opsonizing activity, they also stimulate the complement system. In protochordates and agnathans, the lectin pathway is characterized by MASPs and MBL (Endo et al. 2006; Ourth et al. 2008). The existence of isoforms boosts the diversity of lectins. In the Japanese eel, FTLs are present in at least seven isoforms and produced in the gill, intestine, and liver (Honda et al. 2000). It was revealed from the eel agglutinin structure that more sequence variations among isoforms were present in regions that contact the carbohydrate ligand. This suggests slight dissimilarity in sugar specificity, which could enlarge the range of possible surface ligands (Bianchet et al. 2002). Thus, the diverse lectins in fish would enable comprehensive identification of non-self-epitopes and destroy the pathogens. In teleost fish such as carp, zebrafish, and salmonids, numerous MBL isoforms have been recognized as Ca^{2+}-dependent opsonin. They are revealed solely in the spleen and liver (Nikolakopoulou and Zarkadis 2006).

Several studies suggest that acquired immune constituents in fish decrease their functionality at less temperature, and innate immune constituents are less affected. Therefore, innate immune reactions are the choice for the generation of high disease resistance. Hence, various lectins in the immune reactions rapidly identify and neutralize the pathogens and lead to an effective long-term adaptive immunity (Ewart et al. 2001; Magnadóttir et al. 1999).

9.4.1 Lectins in Pathogen Recognition

The innate immune system defends the host via recognition of microbial targets such as collectins and pentraxins. These identify non-self-antigens through carbohydrates which act as an opsonin and encourage their damage by phagocytic cells and complements (da Silva Lino et al. 2013). Galectins, like CTLs, function as recognition factors of microbial glycans or as regulators of several adaptive immune functions via modulation of B-cell development, induction or prevention of T-cell apoptosis, and modulation of T-cell responses and the activity of macrophages, neutrophils, and eosinophils (Vasta et al. 2011). Several other lectins are known to participate in the identification of pathogens. MBL from Atlantic salmon has also exhibited activity against *Aeromonas salmonicida.* The cobia (*Rachycentron canadum*) ovaries contain a lectin which has an antibacterial effect against *Escherichia coli* (Ottinger et al. 1999; Ngai and Ng 2007).

9.4.2 *Role of Lectins in Hemagglutination and Agglutination of Pathogens*

Agglutination is the process by which cells and viruses adhere together. The substances that induce agglutination are known as agglutinins. Lectins are crucial agglutinins in the immune system. Several lectins agglutinate bacteria as well as non-self-erythrocytes (hemagglutination). After the agglutination of pathogens, they are targeted for destruction through complement activation, phagocytosis, or lectin-mediated degradation. This mechanism is crucial for preventing pathogen uptake, especially in mucosal surfaces. On the skin, this mechanism can allow the pathogen to fall off during swimming and in the gastrointestinal tract the pathogen is ejected along with fecal matter. Agglutination can be inhibited by heating and can be Ca^{2+} dependent (Boes 2000). Lectins that contain one CRD should form dimers or higher structures for agglutination (Mu et al. 2017; Kugapreethan et al. 2018).

Some examples of lectins exhibiting agglutination are galectin-1 (Atlantic cod), galectins congerins (*Conger myriaster*), pufflectin (*Takifugu rubripes*), galectin AJL-1, AJL-2 (Japanese eel), SsLTL (*Sebastes schlegelii*), rOnMBL (*Oreochromis niloticus*), CsLTL-1 (*Channa striatus*), and a C-type lectin (*Sebastes schlegelii*) (Brinchmann et al. 2018).

Pufflectin that binds with *Heterobothrium okamotoi* shares homology with MBL from plants instead of other animal lectins. It can be seen revealed in esophagus, gills, skin, oral cavity wall, and intestine. It is considered to be of defense against parasites as it has no effect on several pathogenic bacteria tested (Tsutsui et al. 2003).

Hemagglutination can be seen in MBL from flathead (*Platycephalus indicus*) and other MBL's like SsLTL (*Sebastes schlegelii*) and natterin (*Gadus morhua)* (Kugapreethan et al. 2018; Rajan et al. 2017; Tsutsui et al. 2011). Hemagglutination and hemagglutination inhibition can be seen in Fig. 9.3.

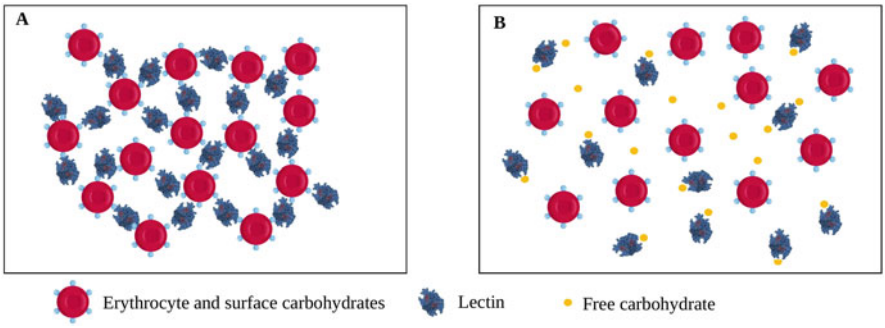

Fig. 9.3 Scheme of hemagglutination and inhibition of hemagglutination. (**a**) The lectin induces hemagglutination of the erythrocytes by linking to the erythrocyte surface carbohydrates. (**b**) Hemagglutination inhibition can be observed when the lectin binds to free carbohydrates

The attachment to an infectious agent is not connected to killing or growth inhibition as seen in the case of congerin isolated from conger eel. It induces the *Vibrio anguillarum* agglutination but not its killing or growth inhibition (Kamiya et al. 1988).

9.4.3 Lectins in Phagocytosis and Endocytosis

Lectins are known to give protection to the host against diseases, promote infections, and help pathogenic organisms. They act as opsonins and promote the phagocytic and endocytic uptake of pathogens. The pathogens exploit this property to gain access to cells. The pathogen uptake serves two functions: killing the pathogen using lysosome and sampling, and enabling the peptides of the infectious agent to be given out to the immune system. Recognition and elimination of an infection via phagocytosis by a dendritic cell can be seen in Fig. 9.4. But pathogens develop several ways to evade the immune system. Some of them are escaped from the phagosome itself, some of them either obstruct the fusion with lysosome or acquire ways to survive inside these acidic vesicles (Cossart and Helenius 2014; Brinchmann et al. 2018).

The serum lectin of salmon is an opsonin for *A. salmonicida*. Phagocytosis of *A. salmonicida* (heat killed) was enhanced in a dose-related aspect by this lectin. Lectin isolated from *Sparus aurata* binds to *E. coli* and stimulates phagocytosis (da Silva Lino et al. 2013).

Globotriasylceramide (Gb3) is the ligand for shiga toxin B subunit, and few serotypes of *E. coli*. GB3 enhance clathrin-dependent endocytosis (Sandvig et al.

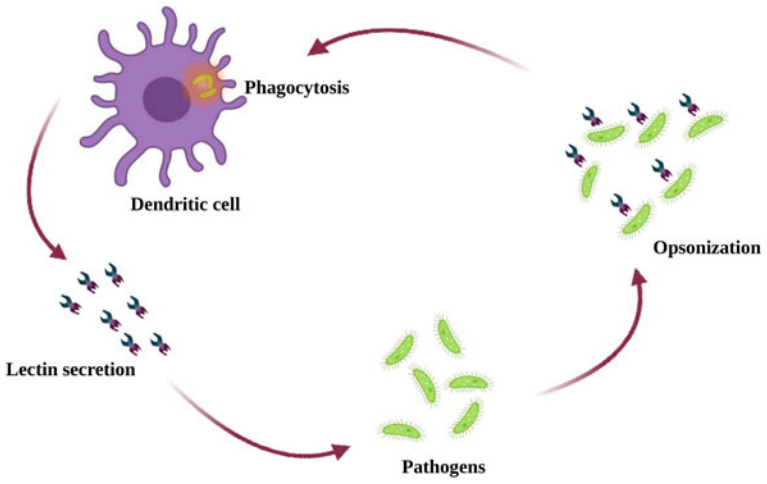

Fig. 9.4 Recognition and elimination of infection by phagocytosis

1989; Watanabe et al. 2009). The promotion of uptake of pathogens by several teleost lectins has been found. The F-type lectin present in *Dicentrarchus labrax* raised fucose-inhibitable and phagocytic intake of *E. coli* with the help of peritoneal macrophages (Salerno et al. 2009). This further suggests that the process of phagocytosis can enhance the liberation of lectins to increase phagocytosis during infections (Rajan et al. 2013).

9.4.4 Lectins in Inhibiting Uptake and Colonization of Pathogens

Pathogen ligands interact with host surface receptors and enter into cells. As a defense mechanism against infection, this interaction can be blocked. The galectins (Drgal1-L2, Drgal3-L1) present in zebrafish bind to IHNV glycoprotein. The entry of the virus occurs at the bottom of the fins. The galectins were observed to be preventing the adhesion of virus to the fish epithelial cells in vitro (Bearzotti et al. 1999).

As discussed earlier, lectins have a dual role. The pathway used for defense can be developed for pathogen intake and access to cells leading to infection. SsLec1 lectin (*Sebastes schlegelii)*, C-type, was found to increase ISKNV. Whereas, SsLec1 effectively phagocytosed and killed bacteria via head kidney macrophages and expression of SsLec1 gene was observed to increase in bacteria-infected fish (Liu et al. 2016).

9.5 Antimicrobial Role of Lectins in Fish

Distinctive microbial motifs that are shared by closely related microorganisms are identified by pattern recognition receptors. It recognizes various microbial constituents such as lipoteichoic acids, β-glucans, lipopolysaccharides, and peptidoglycan. Lectins act as soluble opsonins, phagocytic ligands, and agglutinins (Elumalai et al. 2019).

Lectins can cause either growth inhibition or they kill microorganisms. In most of the assays, the distinction between inhibition of growth and killing is unclear. Hence, the general effect is referred to as "antibacterial." Inhibition of growth can be studied utilizing fluorescent stains (propidium iodide) which stains cells with holes in the cell membrane. To study antibacterial activity, a spectrophotometer could be employed. C-type lectin AJL-2 with *E. coli* exhibits low absorbance (600 nm). This indicates the killing of bacteria. This experiment can be performed at various wavelengths, but 600 nm is commonly preferred as there is less interference from common broths. It measures only turbidity without distinguishing dead and live cells (Tasumi et al. 2002; Brinchmann et al. 2018). An agar diffusion test using mannose-

specific tetrameric lectin isolated from the ovaries *of Rachycentron canadum* against *E. coli* gives a clear area suggesting inhibition of growth.

For in vivo studies, fishes challenged with pathogens exhibited alterations in the lectin gene or protein expression. Several MBL in fish exhibits such alterations upon challenge by bacteria, fungi, and virus, indicating their role in defense against pathogens. The MBLs from Nile tilapia were found to be increased after a challenge with *Streptococcus agalactiae* or *Aeromonas hydrophila* suggesting its role in defense against microorganisms. The mRNA of lily-type lectin (mannose binding), *SsLTL* gene was also observed to be overexpressed in the gill. However, upregulation does not happen with every challenge. On lipopolysaccharide challenge, SsLTL expression was downregulated even though it was minimal (Mu et al. 2017; Kugapreethan et al. 2018). CsLTL-1 was over expressed in the gills of *Channa striatus* after disease caused by bacteria *Aeromonas hydrophila* and the fungus *Aphanomyces invadans*. Since pathogens also take over the host machinery to gain cell entry, it is necessary to study the role of a high quantity of lectin for the protection of the host (Arasu et al. 2013).

Lectin specific for L-thamnose, STL, was observed to recognize lipopolysaccharides and lipoteichoic acid in the eggs of steelhead trout. It also agglutinated *E. coli* K-12 (Tateno et al. 2002). Tetrameric lectin from the ovaries of cobia exerted antibacterial effect against *E. coli* with 50% inhibition (Ngai and Ng 2007). rTc-hsL from Chinese white shrimp (*Fenneropenaeus chinensis*) bound *E. coli* and *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, *B. megaterium*, and *B. thuringiensis*. It also exhibited activity toward *Pichia pastoris*, a methylotrophic yeast species (Sun et al. 2008).

Twelve galectin genes are highly expressed in mucosal tissues of *Ictalurus punctatus*. It was observed that the expression profile of these galectins exhibited tissue and pathogen-type-specific alterations on challenge with Gram-negative bacterial pathogens (Zhou et al. 2016). The congerin I and II present in the conger eel was observed to bind nematodes (*Cucullanus*), which parasitize the abdomen of the host. Galectin-1 is present in Atlantic cod and also in *Francisella noatunensis* infected granuloma cells (Nakamura et al. 2012; Rajan et al. 2013).

Antifungal activity was shown by only few numbers of lectins. They generally bind and agglutinate fungi or their expression level may be upregulated when a fungal challenge arises. The mode of action will be similar to that against the bacteria (Xue et al. 2013; Wei et al. 2010). Ec-CTL from *Epinephelus coioides* demonstrated antifungal activity. It is Ca^{2+} dependent and upregulated when challenged by *Saccharomyces cerevisiae*. It binds to and aggregates the pathogen (Wei et al. 2010). Intelectin with glycan binding receptors was isolated from lamprey (*Lampetra japonica*). It plays an important function in the defense mechanism against *Candida albicans*. Agglutination of the yeast was observed (Xue et al. 2013).

In an in vivo study, it was observed that galectin-1 gene expression in flounder (*Paralichthys olivaceus*) was reduced after the injection of polyinosinc: polycytidylic acid (poly I: C) during the initial period then subsequently it increased. The neutralization of LCDV (lymphocystis disease virus) and the inhibition of cytopathy of the infected cells were performed by recombinant galectins-1, and it

also prevented inflammatory reaction (Liu et al. 2013). RbFTL-3 found in *Oplegnathus fasciatus* which is principally found in the intestine was perceived to protect fathead minnow cells from hemorrhagic septicemia virus infection. A proteomic study of this lectin revealed that the transfected cells exhibited alterations in signaling of thrombin and budding of virus (CCT5, TUBB, and SNF8) (Cho et al. 2014).

Further studies on structure, function, molecular mechanisms, and clinical trials of lectins are required to investigate the toxicity and therapeutic potential of lectins. Evaluation of synergism of lectins with existing antimicrobial agents will contribute to increase knowledge about antimicrobial mechanisms and drug design applications (Elumalai et al. 2019).

9.6 Methods of Study of Lectin–Pathogen Interaction

During the past two decades, there has been an increase in the availability of EST and genomic databases for several organisms. This helped the search for lectin-recognized sequence motifs and the evaluation of the structural variation of lectins. Further perception into their functions was obtained by the use of genetic approaches in model organisms. Bioinformatic tools such as ProfileScan, SwissProt, BLASTP, and PfamA have enabled the recognition of lectins and lectin domains. For example, the presence of diversified lectin repertories containing multiple members of lectins has been revealed when the mining of pufferfish (*Takifugu rubripes*) and zebrafish (*Danio rerio*) genomes were conducted. High-throughput, automated, novel technologies were developed for analyzing specificities of the proteins of interest for carbohydrates. The use of glycan microarrays were used for the analysis of specificity of lectin which has enabled the concurrent testing of a large number of possible carbohydrate ligands. Frontal affinity chromatography and surface plasmon resonance helped in the quantitative kinetic analysis of interactions between protein and carbohydrate (Anderson and Ingham 2003; Vasta et al. 2011).

Lectins are isolated from tissues, blood, etc. This method requires a huge quantity of starting material, and many of them use recombinant proteins in addition to or instead of isolated protein. A large number of lectins are obtained when recombinant proteins are utilized. There are certain downsides to this method. The lectin of interest maybe toxic to classically genetically modified cells. Since several proteins are posttranslationally modified, there is a necessity to confirm the recombinant protein used and the original one. Some lectins also undergo co-translational transport into the rough endoplasmic reticulum and then to endosomes. A eukaryotic expression system is preferred, and the glycosylation pattern is examined to make sure it is identical to genetically modified portions of the protein to be enable the study of binding specificities (Brinchmann et al. 2018).

Since-cell morphological and histological can be done with the help of antibodies, they are used to study lectin–pathogen interaction in situ. Lectin-specific antibodies were made against each species are ideally used. Also, antibodies against

a lectin from another species are also used. The sensitivity of the antibodies and their specificity should be checked, and this is done by western blotting or mass spectrometry (Saper 2009; Marchalant et al. 2014).

9.7 Lectin Purification and Characterization

The isolation, characterization, and purification of lectins are important for elucidating their basic properties and biological functions. A vast quantity of lectins has been isolated from mucus, gill, serum, plasma, and eggs by partial purification (fractionation dependent using pH or salt such as ammonium sulfate) followed by exhaustive dialysis. Presently, most lectins are purified by affinity chromatography. Other chromatographic techniques such as molecular exclusion and ion exchange chromatography are needed to obtain lectins with high purity (da Silva Lino et al. 2013).

Lectins can undergo hemagglutination, and thus, a hemagglutination assay with human or animal erythrocytes can be used to identify a lectin in a sample such as tissue homogenate or serum. The addition of mono or oligosaccharide could inhibit hemagglutination activity (Coelho et al. 2012). To explain the emergence and development of lectins in various tissues, gene evolution has been investigated. Also, gene expression analysis for the comparison of infected and healthy fish can be done (da Silva Lino et al. 2013).

9.8 Model for Glycoimmunology: Zebrafish (*D. rerio*)

Fish species like channel catfish, nurse shark, and rainbow trout were used to study the aspects of their immune response. Zebrafish as a model organism extends many benefits over others. It has been used for studies on development, oncology, and immunity (Vasta et al. 2004b).

To observe the roles of specific genes in the immune system, several experimental approaches were executed including antisense methodology, expression of recessive, or overexpression of active protein, testing using mutants were also used. These were combined with clinical trials. Both mechanistic and oncogenic features of lectins in immune responses: tilling and mutagenesis screens could be applied to study it (Vasta et al. 2011).

The spatial temporal expression forms of complement components can be inspected using the zebrafish model (hatched larvae) by challenging fish with an infectious agent (Wang et al. 2008). The genome of zebrafish contains activating or inhibiting signaling motifs (NK receptors). Illr (immune-related lectin-like receptors) genes in zebrafish have important purposes in the immune functions since they are expressed differentially in the lymphoid and myeloid origin (Panagos et al. 2006). Genomic Matching Technique was used, and four ancestral haplotypes of zebrafish were discovered. One of these four haplotypes exhibited a *L. anguillarum*

resistance. Genomic analysis revealed that retroviral insertion, duplication, mutation or deletion were the important factors in MBL development. These discoveries support the concept that distinctive MBL variants explain the zebrafish infection resistance or susceptibility (Jackson et al. 2007).

Galectin repertoire of zebrafish was identified and characterized utilizing different means like in silico analysis, characterization, cloning, etc. Two chimera types, few tandem-repeat types and some prototype galectins, were identified. The sequence similarities of the characterized zebrafish galectins with mammalian galectins allowed their categorizing within these galectin groups (Ahmed et al. 2004).

Zebrafish intelectins (zINTLs) were primarily expressed in liver and are extremely increased when *Aeromonas salmonicida* caused the disease. It suggests intelectin's significant role in protection against diseases (Lin et al. 2009). These outcomes illustrate the possibility of zebrafish as a prototype. Hence, the structural, functional, and evolutionary facets of lectins can be addressed in detail (Vasta et al. 2011).

9.9 Conclusion

The study of host–pathogen interactions in fish is significant since numerous pathogens are present in aquatic environments and disease-free fish will be of great worth to the aquaculture industry. As mentioned earlier, even in eurythermal fish, acquired immune response is diminished at lower temperatures. But the elements of the innate immune reactions are less affected by temperature. The enhancement of innate immunity could lead to more disease resistance. High expression of antimicrobial peptides, alteration in production of antibodies, nutritional enhancement, and reproduction of resistant strains should also be included in research. Thus, leading to higher production and profit gain in the aquaculture industries and will reduce the use of antimicrobial agents. In the future, transcriptome and genome projects on different fish models will disclose the extent of the full lectin repertoire and their participation in recognition and effector functions in innate immunity but also indirectly act as regulators of adaptive immune response mechanisms.

Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 10
Immune System in Fish and Role of Lectins During Infection

B. S. Subi and S. Shabanamol

Abstract As vertebrates, fishes possess two types of immunity named innate and acquired immunity. The innate parameters play involves significantly in fish immunity and disease resistance. Due to the absence of specialised lymphoid organs, the fish immune system is said to be primitive type and constitutes lymphoid organs and immune cells. It is a non-specific mechanism and functions by complement pathway depending on the target recognition by lectins. Lectins are chemically proteins that can bind to carbohydrates, glycolipids, peptidoglycans, glycoproteins, etc. Major types of fish lectins are galectins, C-type lectins, F-type lectins, rhamnose binding lectins, etc. Major roles played by different lectins include recognition of pathogens, agglutination, opsonisation, complement activation, and phagocytosis. Other than these, molecular functions like splicing of RNA, folding and trafficking of proteins also regulate proliferation of cells. Acquired immunity is the specific immune response acquired during life. It involves humoral immunity, cell-mediated immunity, and immunological memory. Even though the adaptive response in fishes is found to be late, it is considered as important for long-lasting immunity and effective vaccination property. In this chapter, fish immunity is discussed, giving emphasis on lectins and its classes involved during the time of infection.

Keywords Fish lectins · Innate immunity · C-type lectins · Toll-like receptors · PAMPs

Abbreviations

AMP	Antimicrobial peptides
Cnx	Calnexin
CRD	C-type carbohydrate recognition domain

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CRP	C-reactive protein
Crt	Calreticulin
CSF	Colony-stimulating factor
CSF1R	Colony-stimulating factor 1 receptor
CTL	C-type lectins
CTLD	C-type lectin like domain
DAMP	Danger-associated molecular pattern
EGCs	Eosinophilic granular cells
FP	Female protein
FReD	Fibrinogen-like domain
FTL	F-type lectins
GALT	Gut-associated lymphoid tissue
GIALT	Gill-associated lymphoid tissue
IFN	Interferon
ILN	Interleukin
IPNV	Infectious pancreatic necrosis virus
LPS	Lipopolysaccharides
LRRs	Leucine-rich repeat containing protein
MAC	Membrane attack complex
MBL	Mannose binding lectin
MHC	Major histocompatibility complex
NCCs	Non-specific cytotoxic cells
NO	Nitric oxide
PAMPs	Pathogen-associated molecular patterns
PAPC	Professional antigen-presenting cell
PRR	Pattern recognition receptors
RBLs	Rhamnose binding lectins
RIG	Retinoic acid inducible gene 1
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SALT	Skin-associated lymphoid tissue
SAP	Serum amyloid protein
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
WSSV	White spot syndrome virus
zITLN	Zebra fish intelectin

10.1 Introduction

Lectins are a diverse group of proteins which can bind to carbohydrate molecules with considerable specificity. These proteins are present in virus, bacteria, cyanobacteria, yeast, plants and animals. The word Lectin originated from Latin *lager*, which means 'to choose' or 'select' and was declared by William Boyd and Elizabeth Shapleigh in 1954. In earlier times, studies on lectins other than plant lectin were seldom because lectins from other sources were not isolated. But in the late twentieth century, the field of glycobiology had a major breakthrough due to the recognition of the lectin preference in animal tissue by the scientific community. The first mammalian lectin was identified in rabbit liver by Stockert et al. in 1974, and also, the first serum lectin was identified by Ashwell and Monter in 1974. Lectin agglutinates cells and precipitates polysaccharides, glycoprotein or glycolipids. They are also carried out in various cellular functions like apoptosis induction, cell to cell interactions and also in antimicrobial activities especially antibacterial and antiviral activities. They are also reported to possess anti-proliferative activity for cancerous cells, mitogenic activity and antitumor activity. The lectin–sugar association is a primary event in some of the biological processes such as infections, immune reaction, inflammation (Bouwman et al. 2006). In addition to this, they possess significant role in cell-to-cell recognition and cell signalling (Gabor et al. 2004).

In fishes, distribution of lectins is found to be present in intracellular and extracellular area, mucosa, serum, etc., and the lectin from mucosal surfaces puts forth its role in defence mechanism of mucosa. Like mammals, the eyelid, nose, mouth, bladder and gastrointestinal tract are also found to be such area in fishes. In addition, fish kin and gills also contain mucosal surfaces. As the fishes are in constant exposure to water, the mucosal tissue on the surface comes in close contact with different microorganisms and parasites and also with other abiotic factors which make them infection prone. This forms a risk and needs to maintain its health and homeostasis. Parasites usually cause many digestive disorders in fish as follows. The protozoan parasites, *Spironucleus* and *Hexamita* parasitise the intestines of fish groups, viz. cichlids, bettas and gonius. Dactylogyrus commonly infects fish gills. In case of bacterial infection commonly known as varieties are *Aeromonas* spp., *Pseudomonas* spp., *Flavobacterium* spp., *Acinetobacter* spp. and some of the gram-positive bacteria like *Lactococcus garviae* and *Streptococcus iniae*. Water and sediments together act as the reservoir of pathogenic bacteria for fishes. The viral infection in fish was also predominant. Most of the fish pathogen includes Rhabdoviruses, Buna virus, Herpes virus, Irido virus, Reo viruses, Orthomyxoviruses and Retro viruses. In the case of fungal infections in fish, it attacks mostly the external tissue, but certain mycosis will infect the fish internal organ system. The most common fungal disease in fish are saprolegniasis, branchomycosis, epizootic ulcerative syndrome and ichthyophonius. The fungus genera causing fish infections are *Saprolegnia, Achyla, Aphanomyces, Calypratheca, Thraustotheca, Leptolegnia, Pythiopsis* and *Leptomitus*.

Fishes are susceptible to infection via three major routes such as skin, gastrointestinal tract and gills. The skin mucosal surfaces of fish are said to be the largest mucosal surfaces in fish. The health status of aquatic organism is very much dependent on the environment they live, which includes greater load of microorganisms. Majority are saprophyte, few are pathogenic, and these two categories will infect fishes under favourable conditions. Under normal condition, fish maintain a healthy status using a repertoire of innate and specific defence mechanisms (Ellis 2001). At every moment, the microbes try to cause infection in skin and invade the internal organs in fish. They defend the infection by mainly two mechanisms like innate immune and adaptive immune system. The former one is otherwise called natural immunity which is non-specific and constitutes a sequence of cellular and humoral mechanisms, and the later one is the acquired otherwise the specific immune system performed by the humoral immune response mediated by antibody production and the T-lymphocytes operated cellular immunity. Unlike the specific immune system, the innate or no-specific immune system is not capable of keeping memory and recognising the foreign antigens. Anyways this phenomenon is very crucial in species Pisces, since the production of antibodies in fishes is comparatively lower to that in higher vertebrates. In case of adaptive immunity, the antibody production takes considerable time to get activated and is greatly depending on temperature in ectothermic vertebrates (e.g. in salmonids, the time taken to produce antibody is about 4–6 weeks).

10.2 The Innate Immune System of Fish

The primary line of defence in fish is the skin and mucus membrane. When the pathogenic microorganism enters the host, the cellular and humoral innate response mechanisms were activated (Magnadottir 2006). In fish, dermis, epidermis, scales, mucus and epithelial layer of gastrointestinal tract of fish skin forms the first set of defence, i.e. physical barrier. The main function of this system is to protect the fishes from physical damage and supporting to maintain homeostasis, particularly keeping a minimal exchange rate between fishes and environment. Besides their role in defence, it is considered as a transport epithelium (Shephard 1994). Mucus of fish consists of predominantly glycoprotein which prevents the colonisation of foreign bodies. A number of biological components including lectins contributes to the antimicrobial activities of substrates provided by the mucus layer (Newak 1999; Nagashima et al. 2001). Figure 10.1 shows an outline of fish immune system.

The innate immune system is categorised into three defence mechanisms.

1. Physical barriers.
2. Cellular components.
3. Humoral responses.

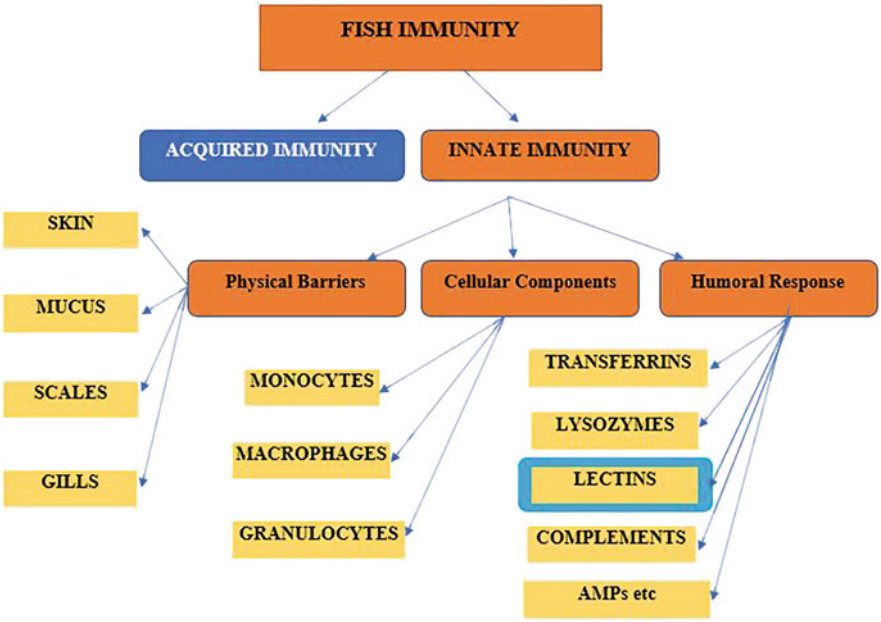

Fig. 10.1 Outline of fish immune system showing the components of innate immune system

10.2.1 Physical Barriers

In teleost fishes, the SALT cells (Skin-Associated Lymphoid Tissues) comprising secretory cells (e.g. goblet cells), lymphocytes (B cells and T cells), granulocytes, macrophages and Langerhans-like cells are present. In a majority of teleost fishes, the dermis layer called leptoid scales characterised by bony scales can be seen. It is reported that catfishes, which belong to teleosts, exhibit loss of scales during evolution and have gained bony dermal plates as a covering of the skin (Liu et al. 2016). In cartilaginous fishes also, the skin may contain melanocytes, macrophages, lymphocytes and granular leukocytes. The scales of these types of fishes are called as placoid scales or denticles. Meanwhile, the lobe-finned fish skin is found to possess keratinocytes, granulocytes and B cells. Mucus secretion by fish skin also acts as both a physical barrier, by entrapping pathogen, and a chemical barrier (Esteban 2012). Similarly, the mucus secretion by teleost fishes also contains a mix of compounds functioning in pathogen neutralisation. The gills of fishes' act as a physical barrier providing immunity in addition to its normal functions. The physical barriers in gills are found to be gill epithelium, glycocalyx and mucus layer as mentioned. The distribution and functioning of gills in teleost fishes as well as in cartilaginous fishes are different. In the former case, the inter branchial septum is in teleost fish, the interbranchial septum is decreased in size with a monocaudal opening at the operculum and does not contain multiple openings. In cartilaginous

fishes, the interbranchial septum supports the gills along their whole length and contains multiple openings of interbranchial septum (Wilson and Laurent 2002). Immune cells such as macrophages, neutrophils and eosinophilic granulocytes are found in the GALT (gill-associated lymphoid tissue) tissues of teleost fishes. It is also found that the microorganisms of the mucosal surface of the gill-associated lymphoid tissue induce antibody producing B cells. The GI tract (gastrointestinal tract) functions in nutrient absorption as well as preventing invasion of pathogens though the epithelium. The fate of a pathogen if it enters into the gastrointestinal system is handled by the entire fish immune system. Unlike mammals, the GAT tissues in mammals are not a tightly organised but contain a network of diffused myeloid and lymphoid cells.

10.2.2 Cellular Components

If a pathogen manages to get beyond the physical barrier, it will be met by the innate immune system's cellular and humoral defences. Monocytes, macrophages, granulocytes such as mast/eosinophil granule cells, neutrophils, dendritic cells and natural killer cells are among the cellular components found in fish. The front (or head) kidney and thymus are the primary sites for leucocyte generation in bony fish, whereas the epigonal organ, Leydig organ, thymus and spleen are the primary sites in cartilaginous fish (Icardo et al. 2012). When a pathogen is encountered, an innate immune cell will recognise a pathogen-associated molecular pattern (PAM) in the pathogen. Once recognised, the innate immune system becomes activated and participates in a variety of responses, including phagocytosis and subsequent pathogen destruction, production of various cytokines, and activation of the adaptive immune system through antigen presentation and cytokine stimulation, depending on their cell subtype.

10.2.2.1 Macrophage/Monocytes and Neutrophils

The first cells to come and respond to an infection in fish are macrophages or monocytes. The colony-stimulating factor 1 receptor regulates the differentiation of vertebrate macrophages (CSF1R). Macrophages are vital in inflammation and pathogen infections, and they play a role in both the innate and adaptive immune systems. Pathogens are destroyed by macrophages from several types of bony fish in the innate immune system through phagocytosis. Macrophages serve as professional antigen-presenting cells (PAPCs) in the adaptive immune system, presenting phagocytes and receiving T lymphocytes from the adaptive immune system through a process known as antigen presentation.

Neutrophils are the general granulocytes in long fish, and they, like macrophages, act in the innate defence against infections. They have powerful antimicrobial responses through a variety of intracellular and extracellular pathways, including

the release of cytotoxic and antimicrobial enzymes in granules. The size, shape and staining qualities of granulocytes in cartilaginous fish are used to divide them into three categories. They are G1 granulocytes, most common granulocyte; G2 granulocyte, resemble mammalian eosinophilic granulocytes; G3 granulocyte, more commonly seen in cartilaginous fish compared to bony fish.

10.2.2.2 Recognition of Non-self

The innate immune response is activated when germline-encoded intracellular or extracellular pattern recognition receptors (PRRs) of immune cells bind to a pathogen-associated molecular pattern (PAMP) found on pathogens such as bacteria-derived LPS, viral RNA or bacterial DNA, or a danger-associated molecular pattern (DAMP) found on protein or other biomolecules related to stressed or injured cells. All PRRs have a domain that interacts with downstream signalling molecules in order to recognise PAMP on a regular basis. PRR in mammals are classified into five major groups like:

(a) Toll-like receptor (TLR).
(b) Retinoic acid inducible gene I (RIG-I)-like receptor.
(c) C-type lectin receptors (CLRs).
(d) The nucleotide boundary domain.
(e) Leucine-rich repeat containing proteins (LRRs).

Many homologues of mammalian PRRs were also identified in fish. Of these, the first PRR identified in fish was TLRs. Over 20 TLRs have been identified in different fish species (Kawai and Akira 2009).

10.2.3 Phagocytosis

Phagocytosis is one of the oldest and most widespread defence mechanisms against foreign substances. Even unicellular eukaryotes, which predate sophisticated multicellular life, have this mechanism. When a pathogen binds to a PRR, phagocytes such as macrophages, monocytes, neutrophils and dendritic cells, which are found in both bony and cartilaginous fish, undergo phagocytosis. Following engulfment, the pathogen-containing phagosome joins to a lysosome to create a phagolysosome, where the pathogen is destroyed in a variety of ways, including the formation of reactive oxygen species (ROS) and nitrogen oxides (NO) (nitric oxide) (Newman et al. 2001).

10.3 Humoral Responses

Generally, different macromolecules in the cells mediate the humoral responses which are released into the humoral components in fish complement system such as lysozyme, anti-microbial peptides, acute phase protein.

10.3.1 Complement System

The complement system is an important part of the body's natural defence against infections. Complement is a system made up of around 30 proteins that can be found in plasma and on all surfaces. This collection of proteins is organised into a ladder of proteolytic cascades that begin with the detection of pathogenic surfaces and progress to the production of potent pro-inflammatory mediators (anaphylatoxin), opsonisation (coating) of pathogenic surfaces via the assembly of membrane attack vesicles (MAC). Classical, lectin and alternative routes are the three major pathways that activate the complement system. The first cells to come and respond to an infection in fish are macrophages or monocytes. The colony-stimulating factor 1 receptor regulates the differentiation of vertebrate macrophages (CSF1R). Macrophages are vital in inflammation and pathogen infections, and they play a role in both the innate and adaptive immune systems. Pathogens are destroyed by macrophages from several types of bony fish in the innate immune system through phagocytosis. Macrophages serve as professional antigen-presenting cells (PAPCs) in the adaptive immune system, presenting phagocytes and receiving T lymphocytes from the adaptive immune system through a process known as antigen presentation. C3 is fragmented to C3b, an internal thioester link is exposed, allowing C3b to covalently connect to hydroxyl groups on nearby carbohydrates and proteins. This action underpins the entire complement system by effectively identifying microorganisms as alien, leading to increased complement activation of the surrounding opsonised surfaces, culminating in anaphylatoxin synthesis and MAC assembly (Walport 2001a, b).

The lectin route is an immunoglobulin-independent form of function. Rather than recognising antigen-antibody immune complexes, the lectin pathway conducts non-self-recognition through germline-encoded pattern-recognition receptors (PRRs) such as mannose-binding lectin (MBL) and ficolins. The adaptive immune system's antigen recognition receptors (e.g., antibody, T-cell receptors) potentially have the ability to recognise any antigen due to their tremendous somatic diversity. PRRs, on the other hand, concentrate on a few highly conserved structures seen in a vast range of microorganisms known as pathogen-associated molecular patterns (PAMPs). A good-characterised receptor of the protein family collectin is the MBL and is called so due to the fusion of one collagenous domain with a calcium-dependant domain of lectin produced in liver later secreted into the plasma as a part of acute phase response. On gram-positive and gram-negative bacteria, yeast, viruses, and parasites,

MBL binds with generic carbohydrate PAMPs. MBL forms complexes with Mannose Binding Lectin associated serine proteases (MASPs) - 1,2 and 3. They are functionally and structurally similar to C1s and C1r also in the sameway the MBL binding to pathogenic surfaces cause associated MASPs to activate,cleave C2 and C4, and ultimately generate C3 convertase in both the classical and Lectin pathways (Takahashi et al. 2008). C4bC2a is a surface-bound C3 convertase that converts inactive C3 to C3a, an anaphylatoxin that works as a chemotactic factor and aids in inflammation, while C3b acts as an opsonin. Through the sugar binding domains like mannose and *N*-acetyl glucosamine the MBL recognises the foreign particles. The cell wall of fungi is rich in mannose, while peptidoglycan of gram-positive bacteria contains large amount of *N*-acetyl glucosamine, making these microorganisms ideal substrate for the lectin pathway. IgA is a mannosylated immunoglobulin and an important intermediator in mucosal immunity which triggers the lectin pathway and is thought to act coordinatedly with MBL in defence (Roos et al. 2001).

The spontaneous activator of C3 in the alternative pathway is enhanced by the covalent binding of C3 (H_2O) to various microbial surfaces (i.e. various bacteria, fungi, parasites). This newly found C3 (H_2O) is able to interact non-covalently with factor B (Bf). Then the factor D (Df) present in the plasma breaks down into Ba and Bb fragments, leading to the formation of the alternative C3 converter. This is short-lived and can split many surrounding C3 molecules into C3b and C3a. If C3b is close to an activating surface, C3b can be covalently attached to the surface through its exposed and highly reactive thioester bond. So an additional C3 convertase is formed by the bound C3b, leading to the C3 cleavage amplification suddenly resulting in the huge deposit of C3b molecules into the activating surfaces. The freshly synthesised C3b bind to the existing C3 convertase and form the alternative C5 convertase, which have the power to cleave C5, leading to the subsequent assembly of the membrane attack complex (MAC) (Muller-Eberhard 1986).

10.3.2 Lysozyme

The enzyme lysozyme can be found in fish mucous, serum and eggs. It is capable of breaking down the peptidoglycan layer of bacterial cell walls. It is a leukocyte-secreted enzyme with a wider range of activity in fish than in mammals. Lysozyme synthesis takes place in neutrophils. As a result, fish lysozyme is mostly found in leukocyte-rich organs, particularly the head kidney, and antigenic invasion sites such as cells, the skin, the gastrointestinal system and eggs. It is also found in freshwater and marine fishes' body mucous, peripheral blood and other tissues. It attacks the lipopolysaccharide layer of bacteria and damages the outer cell membranes, and it allows reaching and injuring deeper structures. The increase in permeability ends in decline of cell characterised by the absence of lysis. Hence, it is well evident that fish lysozyme has a preferable antibacterial activity than mammalian lysozyme towards gram-negative and -positive bacteria (Yourif et al. 1992). It is bactericidal and kills even the serious pathogens such as *Aeromonas salmonicida and Aeromonas*

hydrophila. The other functions of lysozyme are opsonisation, phagocytosis and complement system activation. One of the most researched inherent components in fish is lysozyme. C-type (chicken type) and goose g-type lysozymes are the two forms of lysozyme found in numerous teleost taxa. They have been detected in neutrophils, monocytes and to a lesser extent macrophages from a variety of organs, including liver, kidney, spleen, gills and mucus. The role of lysozyme appears to be comparable in both bony and cartilaginous fish, according to research.

10.3.3 Antimicrobial Peptides (AMPs) in Fish Immunity

Antimicrobial peptides (AMPs) are a diverse group of highly conserved peptides found throughout nature. Fish are an excellent source of these peptides since they express all of the major AMP classes, including defensins, cathelicidins, hepcidins, histone-derived peptides and piscidins, a fish-specific cecropin family.

10.3.4 Lectin

Lectins are also called as natural agglutinin. It is seen as natural peptides or agglutinins in fish. They are thought to possess significant role in innate immunity by mediating pathogen identification. Apart from its role in innate immunity, it functions in fertilisation, embryogenesis and morphogenesis. In the last section of this chapter, we go through the many types of fish lectins functioning in humoral response of fish immune system.

10.3.5 Non-specific Cellular Immunity in Fishes

Toll-like receptors (TLRs), granulocytes, macrophages and non-specific cytotoxic cells are among the leukocytes implicated in fish's innate non-specific cellular immunity (NCCs). They are tiny protein molecules that recognise microorganisms' transformed compounds. Macrophages and granulocytes circulate in the blood and secondary lymphoid tissue, where they have functions in the inflammatory response, which is the cellular immune response to invaders or tissue damage. Eosinophilic granular cells are a type of slow granulocyte that targets parasite infection (EGCs). They are the innate cellular immunological response of the host to helminthic infestation at mucosal sites like the gut and gills. NCCs also target protozoa and viral infection of host cells, causing them to emerge in mucosal areas, blood circulation, and lymphoid tissue. Apoptosis and necrosis were the main mechanisms driving their destructive ability. Because innate immunity lacks pathogen specificity, a high number of innate immunity cells act swiftly. Non-specific innate immunity,

unlike the particular immune system, lacks a memory component. As a result, exposure to a similar pathogen does not result in a greater or faster secondary immune response. Stafford et al. (2003) in goldfish were the first to report TLRs in fish, a significant part of fish innate immune system. TLRs in fish recognise the distinctive transformed molecules of microorganisms known as pathogen-associated molecular patterns, just as they do in mammals (PAMPs). This detection triggers an anti-inflammatory response, which kicks off the body's natural defences. Non-specific cytotoxic cells are cells that look like mammalian natural killer cells but are found in fish (NCC). They share a lot of commonalities, including the competent lytic cycle, the largest cell for lyses, target cell recognition, and the desire to lyse infecting microorganisms. But the differences they perform were in the killing dynamics and also the target cell morphology and specificity. They are reported to possess highest activity in the kidney head of teleost fishes.

10.3.6 Alkaline Phosphatase

In fish, alkaline phosphatase is also an important enzyme. It is a lysosomal enzyme found in different human secretions, including body mucus, intestinal mucus and blood serum (Nigam et al. 2012). AP levels rise during parasite infection, and it is a key enzyme in the fish innate immune system.

10.3.7 CRP or C-Reactive Protein

The most primitive protein to be found in plasma of human and a greater number of animals due to damage to tissues, inflammation and also infection is the CRP. It is a protein that comes under pentraxin family. It is found to react with the C-polysaccharide of *Pneumococcus* bacterium, so named C-reactive protein. The major source of CRP was listed as smooth dogfish, and CRP has been isolated from smooth dogfish, *Mustelus canis*, Japanese eel, Channel fish, Tilapia and Murrel fish. In teleost fish, CRP increases in serum when exposed to endotoxin of bacteria. It possesses lectin like agglutinating properties and acts as an opsonin to facilitate phagocytosis and complement activation in the studies done in marine fish pathogen *Vibrio anguilar* sp. (Nakanish 1986).

10.3.8 Transferrin

Transferrin is a multifunctional protein based on the activity of bilobed monomeric glycoproteins that bind to iron and is functioning in iron metabolism associated with the innate immune response. Its function is to safely move iron prior to absorption

and use from storage sites in the body. Because too much free iron is harmful to cells, mild management of iron metabolism keeps the positive and toxic effects in check. Transferrin is produced in the liver and circulated in the bloodstream, but it is also found in the brain and central nervous system, as well as the testes, ovary, spleen, mammary gland, and kidney. Transferrin's job is to bind to iron and create a low-iron environment in which few bacteria can survive and pathogenic germs' infectivity is restricted. It has been found in nearly all fish species, including the cat shark, Scyllium stellare, Lemon strout, Lamprey, and Pacific hagfish.

10.3.9 Interferon in Fishes

Interferon is a cytokine that various cell types produce in response to viral infections. It has the ability to boost the host cell's resistance to certain viruses by promoting the synthesis of proteins that prevent viral mRNA from being translated. IFN are species-specific in teleost fishes. IFN protects other cells from viral infection by attaching to different receptors, resulting in the activation of hundreds of genes. Infectious pancreatic necrosis virus (IPNV) infects Atlantic salmon cells, and IFN-1 has significant antiviral effect. IFN activity has been seen in rainbow trout, salmon and halibut, among other fish species (Robertsen 1999).

10.3.10 Interleukins

Interleukins are cytokinin molecules that play a role in the immune system's intercellular control. They're also in charge of bathing infected areas in granulocytes and macrophages, as well as secreting more cytokines to cleanse the area. A total of 35 ILN families have been recognised (Secombes et al. 2011). L-B homologues have been found in nearly 13 teleost species. For advanced viral pathogens, it has a conserved function of macrophage movement and T-cell recruitment. As a result of infection, IL-A and B are frequently the first to be created, and many people continue to manufacture interleukin for increased specificity to the inflammation. When an antigen is presented to the fish adaptive immune system, IL2 enhances the T-cell replication. IL4 is known to possess efficiency in promoting differentiation of TH2 (Zou and Secombes 2011).

10.3.11 Other Cytokines and Chemokines

Other cytokines, such as IL6, were involved in the cascade leading to an inflammatory response for gram-negative bacteria, in addition to TNF and IL1. Other cytokines involved in leukocyte differentiation found in fish include

granulocyte colony-stimulating factor (CSF), macrophage CSF, and IL-7. Chemokines, on the other hand, have been discovered in some fish species (Uribe et al. 2011).

10.3.12 Natural Antibodies

Without any antigenic stimulation, antibodies are naturally produced in fish called natural antibodies. They always show high amounts in fish serum on pathogen invasion. They provide immediate and broad protection from different types of pathogen. So it is found to be a key component in innate immunity. These antibodies were linked to adaptive immunity too.

10.4 Adaptive Immunity

As the invader got a chance to survive after crossing the physical barrier and specific innate immunity, then the third line of immunity starts to work that is the specific adaptive immunity. It is composed of highly specialised, systemic cells and features to culminate the further activities of pathogens or by resisting its entry. Antibodies and lymphocytes, sometimes known as humoral and cell-mediated immune responses, are the two key components (Uribe et al. 2011). Pathogens are destroyed by antibodies in humoral immunity. Pathogens are destroyed by T cells, phagocytosis, and toxins in cell-mediated immunity. Adaptive immunity cells are T cells and B cells. The 'memory' which distinguishes adaptive immunity from innate immunity is a critical component.

10.4.1 Humoral Immunity in Fishes

Humoral immunity includes substances found in body fluids or juices that include the neutralisation of pathogens and toxins, complement activation, promotion of opsonin phagocytosis and elimination of pathogens. The humoral immune response is mediated by secreted antibodies produced by the B lymphocyte line or by B cells. These are transformed into plasma cells that produce secret antibodies. CD4+ T-helper cells provide costimulation that supports their entire process and allows secreted antigenic antibodies to bind to antigen on the surface of the invading microorganism and dispatch it for destruction. Immunoglobulin in fish is found in skin mucus, intestines, gill mucus, bile and systematically in blood plasma. Three classes of antibodies have been identified in both bony fish and cartilage fish, IgM, IgD and IgZ/T in bony fish and IgM, IgW and IgNAR in cartilage fish. IgM, IW and

IgN have been identified in lung fish, but two forms of IgW have been identified in Coelanthus.

In all jawed vertebrates, IgM stood as a conservative one. It is a tetramer and had eight antigen-binding sites. But one exception is there, that is, on coelacanths it is absent. Both secretory and transmembrane versions are detected. In all jawed vertebrates, it plays a comparable role in triggering opsonisation, antibody-dependent cell-mediated cytotoxicity and complement activation. It also takes part in both innate and cell mediated immunity. IgM in teleost fish is a tetramer and monomer in some other fishes. IgD is found in teleost fishes. IgD is found in both trans membrane form and secretory form. It is phylogenetically as old as IgM. IgD is the second immunoglobulin isotype identified in fish. IgW is found mainly in cartilaginous fish. Different studies reported that the amount of antibodies in fish serum depends on temperature and water quality they live.

10.4.2 Cell-Mediated Immunity

A special receptor known as T-cell receptor is always in charge of initiating a response to an antigen (TCR). CD4+ is a surface marker found on Th cells. It is a trans membrane glycoprotein that acts as an effector cell or a memory cell to coordinate the immune response. They are required for both natural and vaccine-induced immunity to be triggered and maintained. T-cell receptor system and major histocompatibility cells (MHC) were discovered in teleost and elasmobranches. MHC I and MHC II are the two classes encoded by the MHC. They are structurally and functionally unique glycoproteins that deliver antigenic peptides to T cells, triggering a distinctive immunological response. MHC class I molecules mainly present peptide molecules derived from cellular proteins for CD8+ Tc cells (Bjorkman and Parham 1990). MHC classes I and II have been found in cartilage and lobular fin fish, but jawless fish and invertebrates lack the MHC gene. From studies on bony fish, it is clear that class I loci are not associated with class II loci, but are found in different linkage groups. Otherwise, it is clear that the classical MHC does not exist in fish. Fish with important unrelated histocompatibility genes have the ability to endow their offspring with a large number of genotypes, increasing the chances of survival (McLarney 1987).

10.4.3 Immunological Memory in Fishes

Before a second encounter to an antigen, fish normally establish a memory response. After being exposed to the same antigen, rainbow trout responds to inadequate amounts of both types of T cells in an antigen-dependent and -independent manner, according to a study. It is noteworthy that the fish responds to the second dose of T-dependent antigens after two exposures, whereas the T-independent antigen only

requires one exposure. The number of antigen-specific B cells in the spleen is exactly proportional to the frequency of B-cell-specific antigen precursors if the reaction is faster and higher in magnitude than the main response. So from these studies, it is clear that the secondary response is caused by the expansion of the memory B-cell pool, rather than by the specific difference in antibodies (Kaattari 1992).

Fish develop a memory response before a second exposure to an antigen (Arkoosh and Kaattari 1991). Rainbow trout respond to suboptimal doses of both T lymphocytes in an antigen-dependent and independent manner after an initial exposure to the same antigen (Arkoosh and Kaattari 1991). It is remarkable that it takes two exposures before the fish responds to the second administration of T-dependent antigens, whereas T-independent antigen requires only one exposure. Additionally, while response is faster and of larger magnitude than the primary response, the number of antigen-specific B cells in the spleen is directly proportional to the frequency of B-cell specific antigen precursors. This finding suggests that the secondary response is caused by the expansion of the pool of memory B cells (Kaattari 1992) and not a specific.

10.5 Gene Expression of Various Lectins in Fish During Infection

Fish respond to different infections from different microbes by activating the various innate and adaptive immune mechanisms. It has been already mentioned in the above sessions in detail about the factors taking part in the immune mechanisms. Of these, lectins or haemagglutinins or lectin like molecules plays a remarkable role in innate or acquired immunity. They are actually innate immune system components that have a preference for carbohydrate moieties. Despite the fact that lectin-mediated defensive responses lack the versatility of immunoglobulin, the acute phase response's speed and the vast range of microbial species recognised that a compensatory advantage gives an instant chance to eliminate the pathogen. In other words, it helps to prevent infection to some extent before the whole immunological repertoire is activated. Normally, neutrophils and macrophages react suddenly to a local infection site during a fish infection and are induced to secrete cytokines that control the expression of various acute phase proteins and induce a systemic induction in collectin, fiolin, pentraxin and other types of lectins. They have been released into body fluids such as plasma, saliva, mucus and alveolar fluids. Lectins and lectin-like receptors are also normally expressed in a wide variety of tissues and natural killer cells. They are expressed in fish liver through combination with acute phase reactants and eventually enter the bloodstream. By binding to the microbial surface, lectins target potential pathogens for various biological immune responses, such as immobilisation, opsonisation and death by complement components and mediate downstream immune regulatory mechanisms.

Table 10.1 shows selected list of lectins and their biological functions. The classification of lectins is usually based on their conserved sequence motifs and

Table 10.1 Some selected lectins and their biological features and functions (Elumalai et al., 2018)

Sl. No.	Fish species	Lectin	Binding specificity	Mode of action	Target	Lectin family	Organ of expression
1	Rock fish (*Sebastes shlegelii*)	SsLec1	Mannose	Agglutination	*Listonella anguillarum*	CTL	Liver
2	Rock bream (*Oplegnathusfasciatus*)	RbFTL-3	Glycan	Thrombin signalling	Viral haemorrhagic septicaemia virus	RBL	Liver
3	Puffer fish (*Takifugu niphobles*)	Tn pufflectin	Mannose	Agglutination	*Heterobothrium okamotoi*	CTL	Skin
4	Puffer fish (*Takifugu rubripes*)	Tr pufflectin	Mannose	Agglutination		CTL	Skin
5	Zebra fish	ZITLN1 ZITLN2 ZITLN3 ZITLN4 ZITLN5 ZITLN6	Mannose Mannose Mannose Mannose Mannose Mannose	Agglutination Agglutination Agglutination Agglutination Agglutination Agglutination	Gram +ve and Gram −ve bacteria	Intelectin Intelectin Intelectin Intelectin Intelectin Intelectin	Spleen and liver Intestine Liver Throat Brain, heart, eye, liver
6	Tilapia fish (*Oreochromis niloticus*)	rOnGal2	Mannose	Phagocytosis	*S. agalactiae*	Galectin	Gills, thymus, head kidney, spleen
7	Conger eel (*Conger myriaster*)	Congerin	β-Galactoside	Agglutination	*Vibrio anguillarum*	Galectin	Skin mucus
8	European eel (*Anguilla anguilla*)	AAA	L-Fucose	Opsonisation	*Vibrio. vulnificus*	FTL	Serum
9	Channel fish (*Ictalurus punctatus*)	IpRBL	Rhamnose	Agglutination and immobilisation	*Flavobacterium columnare*	RBL	Mucus
10	Indian cat fish (*Clarias batrachus*)	CBL	*N*-acetyl glucosamine	Agglutination	Gram −ve bacteria	RBL	Serum

specificity for carbohydrate binding and also the biological process in which they participate (Drickamer and Taylor 1993). They are discussed below in detail.

C-type lectins are calcium-dependent carbohydrate binding proteins that serve as pattern regulation receptors in the innate immune system (PRRs). CTL binding specificity is determined by the carbohydrate recognition domain (CRD) motif, and the tripeptide motifs EPN and QPD bind to mannose and galactose found on pathogen cell surfaces. CTLs are lectins that contain one or more C-type carbohydrate recognition domains (C-type CRDs) that work in a calcium-dependent way. It is also known as the C-type lectin-like domain (CTLD). It can be found in C-type lectin receptors (CLRs). CLRs are generally found as secreted or transmembrane proteins. They interact with pathogens mainly through the recognition of certain carbohydrates as mannose, fucose or glucan structures. Most pathogens express carbohydrates, structures on their surface that act like so-called sugar fingerprints and are recognised by specific CLRs. The recognition of carbohydrates by CLR allows antigen-presenting cells to recognise the major classes of pathogens; H. Mannose only allows the detection of viruses, fungi and mycobacteria. At the same time, fucose structures are expressed by bacteria and helminths and glucan structures are present in mycobacteria and fungi. It is also clear that type C lectins are important recognition receptors (PRRs) that recognise carbohydrate structures. After the binding of the pathogen, CLRs trigger different signalling pathways that induce the expression of specific cytokines that determine the polarisation factor of T cells. Some CLRs induce signalling pathways that directly activate the transcription factor NFKB, whereas CLR influence the signal transmission through the toll-type receiver. Many CLRs such as mannose-binding lectins (MBL), galectins and DC SIGN have been studied by researchers in different fish. Type C lectins have been identified in fish from the earliest jaw vertebrates (sharks) to the more advanced bony fish species such as salmon and carp (Vasta et al. 2004). Most CTL receptors require Ca^{2+} ions to bind, hence the letter 'C' in the name. CTLs are particularly important in the context of immune homeostasis and protection against bacteria, viruses, fungi and parasites. They include signalling pathways that lead to the expression of chemokines and cytokines and lead to phagocytosis (Fig. 10.2).

Agglutination of fungi is usually investigated as part of lectin characterisation, as agglutination can help with clearance by allowing for easier phagocytosis. When challenged with fungus, lectins bind and agglutinate fungi, or their expression level is upregulated, and they also work in a similar manner to eradicate bacteria. When *Branchiostoma belcheri* (amphioxus) is infected with *Saccharomyces cerevisiae*, the C-type lectin is substantially upregulated. By cell wall penetration, the lectin Amphi (CTL) kills *S. cerevisiae* in a Ca^{2+}-independent manner. The lectin reacted with peptidoglycan and glucans, attached to the yeast and killed it directly, according to their findings.

I-lectins, also known as immunoglobulin-type lectins (siglecs), are immune cells that express a protein that interacts with a sialylated glycoconjugate during an immunological response. The CD 33-related siglecs and highly conserved siglecs such siglec 1 (sialo adhesins), CD 22 (siglec 2), MAG, Myelin-associated glycoprotein (siglec4) and siglec 15 are separated into two subgroups in fish. The expression

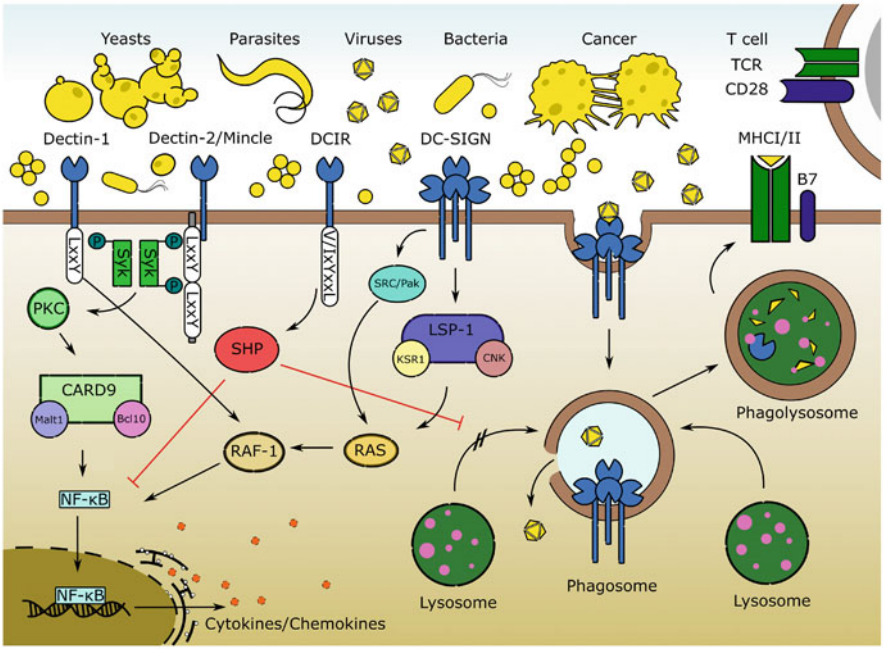

Fig. 10.2 CTL functions and signalling pathways. CTLs recognise molecular patterns of fungal, parasitic, bacterial and viral pathogens (so-called PAMPs) as well as those of dead and malignant cells (DAMPs) (Lindenwalda and Lepenies 2020)

of siglec-expressing genes in distinct lymphoid and non-lymphoid tissue assists in the immunological process, according to studies in salmonid fish and rainbow trout fish. It is a necessary component of both innate and humoral immunity.

Siglec 1 mRNA was found abundant in pikeperch head kidney and spleen and also in spleens and gills of Maraena white fish and rainbow trout. The teleostan head kidney is considered the functional counterpart of the bone marrow in mammals. It contains lymphocytes and macrophages similar to spleen and gills. A recent study by Linghe Kong et al. (2020) in Nile tilapia (*Oreochromis niloticus*) came to an interesting conclusion that siglec-I gene was highly expressed in anterior kidney tissue during *Streptococcus agalactiae* infection. It also seems to be upregulated in anterior kidney and spleen. It was found that *S. agalactiae* showed greater response to the phagocytic activity of macrophages. So siglec-1 is found to have conserved functions of agglutination and promotion of macrophage phagocyte activity on Nile tilapia.

CD 22 is a mature B-cell activation marker. Salmonids maraena head kidney cells had the highest concentration of CD22. The CD22 copy number increases during any microbial infection in fish, indicating a comparable relationship between the CD22 and B cells of the teleostan head kidney.

MAG—The stabilisation of axon myelin contact is aided by MAG. Because they lack myelin, jawless fishes are definitely an exception to siglec-4. In fish, it works as an immunological modulator.

Siglec-15 is the only receptor that is actually conserved from mammals to fish. It is the only receptor from siglec family that encodes both an immunoreceptor tyrosine base inhibitory motif (ITIM) and a charged transmembrane residue that has been found associate closely with the positive signalling adaptor molecules DAP 10, DAP 12 and Fc receptor gamma chain (Angata et al. 2002). In salmonids, high level of siglec 15 was found in spleen (Bornhöfft et al. 2020).

F-type lectins (FTL)—Fucolectins, also known as F-type lectins, were discovered and characterised in teleost fishes (Vasta et al. 2011). The discovery of the FTL family was unexpected and that resulted from the search for fucose binding CTLs in serum and liver extracts from the stripped bass (Morona saxatilis). Because it is made up of a vast number of proteins with larger multiples of the F-type motif, the lectin has a wide range of functional diversity from prokaryotes to amphibians. Some FTLs play a role in immunological detection, while others play a role in microbial pathogenesis, fertilisation and a variety of other roles. FTLs are characterised by a fucose recognition domain (F-type lectin domain (FTLD)), which has a novel fold ('F-type fold'), consisting of a β-barrel with gelatin topology and unique binding of Fucose and calcium sequence motifs, which play a special role in protection against viruses, for example, F-type lectin, RbFTL3, from sea bream (*Oplegnathus fasciatus*), which is expressed mainly in animals in the intestine, could have the ability to produce fatty minnows to protect in vitro against infection by the haemorrhagic septicemia virus (Zhou 2016). FTLs from teleost fish were biologically characterised, revealing their ability to recognise pathogens and their role as opsonin in innate immunity. During teleost fish infections, multivalent FTLDs on FTLs and their particular carbohydrate specificity property aid in the binding of the glycan moiety present on the microbe's surface. Opsonisation is the effect of this. Apart from pathogen detection, FTLs can initiate complement activation, an enzyme-mediated mechanism that can rapidly enhance opsonisation and affect complement in direct killing of a potential pathogen by generating a membrane assault complex. Structural analysis of various FTL isoforms in eels and oysters revealed an important point that FTL can produce exceptional structural and functional diversity for self-recognition or non-self-recognition that is similar to innate immunity (Vasta et al. 2017).

Rhamnose binding lectins were discovered in catfish (*Silurus asotus*), white spotted char (*Salvelinus leucomaenis*) and Spanish mackerel (Scomberomorus niphonius), and a new family of RBLs was discovered in eggs. They play a role in carbohydrate metabolism, polyspermy presentation, crosslinking of carbohydrate-rich proteins of the fertilisation envelope, bactericidal effects, mitogenesis, lectin-mediated cellular cytotoxicity and pathogen opsonisation, among other biological functions. Egg RBLs recognise and bind to lipopolysaccharides and lipoteichoic acid, and agglutinate gram-positive and gram-negative bacteria, suggesting a role in defending the egg against infectious challenges. The presence of RBL in skin mucus supported its proposed roles in immune defence and several articles showed its

participation in various antimicrobial activities such as inhibition of proliferation, cytotoxicity and opsonisation of non-self-cells in particles (Terada et al. 2007). RBLs are divided into five groups based on domain architecture, hemagglutinating activity of human erythrocytes and carbohydrate specificity. In fish it increases in size with the different types of bacteria such as *Staphylococcus epidermidis* in *E. coli* and *Pseudomonas aeruginosa* (Franchi et al. 2011). The RBL transcription profiling study in channel fish (*Ictalurus punctatus*) by infecting *Flavobacterium columnare* as a host pathogen model reported that the RBL expression was induced greater than 100-fold at 3 hr following *F. columnare* experimental infection. But at the same time when RBLs were made saturated with their ligands, it was found that the expression level was lowered and there was decreased tendency of *F. columnare* adhesion.

Pufflectins are found in Japanese puffer fish, *Takifugu rubripes* named as Tr pufflectin. It is known to be the first animal lectin reported to show sequence similarity to some monocotyledon plant lectins. Tn pufflectin was also studied in *Takifugu niphobles* which is closely related to Japanese puffer fish. The speciality of pufflectin is that it has two conserved mannose binding domains. Their genes are mainly expressed in the skin, gills, brain and muscles of teleost fishes. The pufflectin from puffer fish binds to a trematode parasite *Heterobothrium okamotoi* and protect the mucosal tissue from further infection. The expression of their genes in the mucosa suggests that they have a role in maintaining the barrier function of these tissues. It is also a part of the parasite's defence system (Brinchmann et al. 2018).

Intelectin—Since it lacks the normal lectin domain found in all other known lectins, intelectin was dubbed 'Xlectins'. Apart from that, they have a fibrinogen-like domain (FReD) and a particular intelectin region. Although it has been proposed that it has bacteria binding and carbohydrate binding properties, its carbohydrate recognition domain (CRD) has just recently been characterised. The majority of intelectins are located in the intestine. Intelectins have distinct expression patterns in different animals. Intelectin 1 is expressed in tissues of distinct carp subspecies (blue catfish and channel catfish); however, intelectin 2 has diverse expression patterns in different catfish species. In channel catfish, intellectin 2 is expressed in the liver, with low expression in the intestine and kidney, while in blue catfish the expression of intellectin 2 was higher in the head, kidney, liver, heart and also in the intestine, middle kidney and gills. Intellectin expression in grass carp is found in the kidney of the head and spleen and is also expressed in the intestines and gills. In rainbow trout, it is expressed in the gills, liver, intestines and skin. In Zebrafish, intelectin 1 (zITLN1) is highly expressed in the intestine, spleen and liver; intelectin 2 (zITLN2) is mostly expressed in the intestine; intelectin 3 (zITLN3) was mostly expressed in the liver; intelectin 4 (zITLN4) was barely expressed at all; intelectin 5 (zITLN5) has very low expression in healthy zebrafish but high expression in intelectins are proteins that recognise microorganisms and operate as an innate immune response. Tunicate intelectin functions as an opsonin for haemocyte phagocytosis.

Galectins are glycan binding protein that take part in intracellular signalling, cell-cell communication, cellular proliferation and survival. They have at least one carbohydrate recognising domain and found in soluble form. They interact with

the host carbohydrate ligand in embryogenesis and development and with glycan on microbial surfaces (viruses, bacteria, protists and fungi). This allows galectins to function as immunomodulatory pattern recognition molecules in innate immunity. On the basis of the primary structure and polypeptide architecture of the subunits, galectins are classified into 'proto-', 'chimera' and 'tandem repeat' types. Electrolectin is the first galectin identified and characterised by being isolated from the electric eel, Electrophorus electricus. Galectins are synthesised and stored in the cytoplasm, but in case of infection, initiated tissue damage, or after prolonged infection, cytosolic galectins are either passively released by dying cells or actively secreted by activated inflammatory cells. Once exported via a non-classical pathway, the gateways act as PRRs, as well as immunomodulation in the innate response to infection. Gale tins are typically found in poisons where pathogen-induced tissue damage signals arise. One of the most important tasks of gate links is to modulate immunological and inflammatory responses. Activated T and B cells, regulatory T cells, dendritic cells, mast cells, eosinophils, monocytes/macrophages, and neutrophils all express these proteins.

In fish, C-type lectins, galectins and pentraxins have been identified from the earliest jawed vertebrate (sharks) to the more advanced teleost species such as salmon and carp (Vasta and Ahmed 2008).

Galectin 1—The function of galectin-1 is often linked to the reduction of inflammatory reactions. It can help to maintain the balance between Th1 and Th2 immune responses, which are distinguished by the cytokines they generate. It can stimulate the production of anti-inflammatory cytokines like IL-5, IL-10 and TGF in activated T cells while inhibiting the production of pro-inflammatory cytokines like IL-2, tumour necrosis factor (TNF), and interferon-gamma. Galectin-1 interacts with several specific T-cell glycoproteins like CD45, CD43 and CD70. **Galectin-3** is linked to T-cell activation, possibly through interactions with poly-*N*-acetyl lactosamine containing *N*-glycan on the T-cell receptor (TCR). Mast cells, neutrophils and monocytes can all be activated by galectin 3 to produce reactive oxygen species. By operating intracellularly, it has been demonstrated to play a role in macrophage phagocytosis and mediator release cytokinin synthesis by mast cells. These findings point to galectin-3's multifaceted role as a pro-inflammatory mediator and regulator of many elements of the inflammatory response. The galectin rOnGal2 was discovered to have the ability to aggregate a variety of bacteria, including gram-positive and gram-negative bacteria, in a study conducted on Oreochromis niloticus. Similar findings have been reported for various fish galectins. rOnGal2's binding activity against bacteria prevents these pathogens from entering the cell and remaining on the extracellular surface or matrix. This facilitates phagocytosis of bacteria by macrophages.

Pentraxins or pentaxins are a protein family with an evolutionary conserved pentraxin protein domain. It plays a role in acute immune responses. C-reactive protein (CRP), serum amyloid protein (SAP), and female protein are the three members of the pentraxin family (FP). CRP expression has been examined in rainbow trout, catfish, Atlantic salmon, common cod, and dogfish, among other teleost fish species. The level of these pentraxin proteins increased in all of these

fishes when they were injured, traumatised or infected. It is also involved in the immune system, complement pathway activation and the clearance of apoptotic cells. When tissue injury, trauma or infection occurs in teleost fish, the CRP production pattern is shown to vary, meaning it can occasionally rise or decrease. Pentraxins are proteins that recognise a wide range of pathogenic compounds, as well as altered self-molecules and acute phase proteins.

Calnexin (Cnx) and calreticulin (Crt) are two important companions in the endoplasmic reticulum. They are identified from the Chinese woolly crab (*Eriocheir sinensis*) and are known as EsCnx and EsCrt, respectively. They are normally expressed in haemocytes, hepatopancreas, gills and intestines at mRNA and protein levels. The role of Cnxs and Crts in the innate immune system has been studied in crustaceans. When a host is exposed to the white spot syndrome virus (WSSV), Crt is rapidly cleaved off and the host initiates a defensive response against the stimuli. A novel Mjcnx protein has been detected in the cytoplasm and haemocyte membrane of shrimp with turmeric and found to enhance the clearance of *V. anguillarum*. Studies have also been conducted on the Chinese mitten crab *Eriocheir sinensis* and identified rEsCnx and rEsCrt. The lectin showed binding activities of bacteria and various polysaccharides (Huang et al., 2016).

Focalins are fibrinogen-related lectins. They bind to *N*-acetyl glucosamine containing glycan and subsequently activate the innate immune system. It activates lectin complement system for pathogen clearance. They act as an opsonin for pathogen clearance.

10.6 Conclusion

The search for animal lectins are gaining attention to researchers in order to design natural products as new targets for eliciting immune defence. Isolation, identification and functional characterisation of fish lectins play an important role since the antigenic stimulation in fish via lectin production is comparatively similar to warm blooded vertebrates. In different types of fish, actively working innate and adaptive mechanism of immunity is present. This is very much important in evaluating the health status of fish. The importance of lectins in fish during infection is also mentioned in this review, and it gives an idea about how the lectins bind with the pathogen and leads to pathogen degradation processes like opsonisation, phagocytosis, etc. and also in immunomodulation. The review gives an insight to explore the possibilities of developing disease-resistant fishes after molecular level studies of fish lectins and its role in immunological enhancement of fish immune system.

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Chapter 11
Synergistic Activities of Fish Lectins with Other Antimicrobial Agents

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Abstract Lectins consist of a group of protein specifically characterized by carbohydrates binding ability with the carbohydrate recognition domain (CRD) in their molecular structure. These proteins are diverse in nature and found in several organisms consisting of prokaryotes, eukaryotes (plants and animals) and viruses. Several studies are carried out in fish lectins with special emphasis to its antimicrobial capabilities. The research on fish lectins has enhanced new aspects in the fish immunology and lectin biology. A vast variety of lectins were found in aquatic organisms with specific medical uses in recent decades; for example, lectins can be employed in cancer detection and treatment for cell adhesion, cytotoxicity and tumour cell identification. The main characteristic of lectins are the identification of carbohydrates and glycoconjugates in various organs in the animal body, and this property can help as biotechnological tools and in the field of diagnosis, pharmacological and therapeutic applications. Synergistic interactions between natural products and antibiotics could be an effective technique for combating bacterial diseases.

Keywords Fish lectins · Carbohydrate recognition domain · Antimicrobial activity · Nanoconjugates

Abbreviations

CRD	Carbohydrate recognition domain
CTLs	C-type lectins
MBL	Mannose-binding lectin
MDR	Multidrug resistance
MRSA	Methicillin-resistant S. *aureus*

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PRR Pattern recognition receptor
SBL Sialic acid-binding lectin
VRSA Vancomycin-resistant S. *aureus*

11.1 Introduction

Fish lectins are the sugar binding molecules studied from various tissues of different fishes like, Conger eel, Japanese eel, Electric eel, Bighead Carp, Grass Carp, Gibel Carp, Zebrafish, Japanese trout, Atlantic Salmon, Cobia fish, Chinook Simon, Pearl spot, Toxic morey Olive rainbow smelt, Tilapia, Common skate, Pike perch, Steel head Trout, Blue gourami, Potca fish, Spanish Mackerel, and Catfishes like, Channel Catfish, Arabian Gulf catfish, Blue catfish, etc. These lectin comprising tissues are mainly isolated from the eggs, gills, liver, intestine,stomach, electric organs and some of the lectin molecules are from fish skin, serum, and plasma,etc. Lectins from diverse fishes and are different in its number of subunits, molecular weight, amino acid sequences, sugar binding specificity and glycosylation. Also they are having various activities including antimicrobial, antitumor,antiviral immunoregulatory activities (Bun Ng et al. 2015).

In fishes, the pathogenic bacterial strains are particularly important due to the specific protein production may be regulated by temperature related to the upper limit of pathogenecity of bacteria, which is below the optimal temperature of growth (Colquhoun and Sørum 2001). Environmental factors such as pH and osmolarity affect the regulation of gene expression in fish pathogens. The gene expressions well studied by the method of in vivo analysis is an effective method that helps in studying the biological mechanisms through various methods by coupling the gene of interest or its promoter to its reporter gene (Contag and Bachmann 2002). Several studies are carried out in fish lectins and its antibacterial studies against *Edwardsiella tarda* and other bacterial species with a green fluorescent protein as in vivo studies (Ling et al. 2001). Fc-hsL, isolated from a Chinese Shrimp *Fenneropenaeus chinensis*, found in haemolymph has antibacterial activity against the tested bacteria, also against some of fungal pathogens. These have a pattern recognition receptor in the antibacterial defence and acts as an effector in innate immunology of Chinese shrimp (Sun et al. 2008).

The skin of most of the fishes is covered with a mucus layer which acts as a barrier in between the environment and the fish. These mucus layers of skin play an important role in the resistance mechanism against bacterial diseases, which contain mainly lectins and other components. Fish lectins are the proteins that are naturally produced by many species, which has the ability to recognize carbohydrate-binding properties. Lectin molecules take part in various mechanisms including pathogen–host relationship, intercellular communication, immune response by binding specifically to carbohydrates on the surfaces of cells. As a part of innate immunity, lectins are able to involve in increasing opsonization by binding to pathogenic microbes.

Furthermore, many in vitro research studies revealed that lectins are capable to exhibit a bactericidal activity without phagocytic activity while including antibiofilm activity, agglutination with bacteria, disrupting the cell walls of microbes. Some of the studies are summarized, and the potential use of mucus lectins as in vivo antimicrobial agents was evaluated (Taştan and Sönmez 2020).

Several researches investigated antimicrobial effects of the lectins from mucus of various fish skins (Momoh et al. 2014). The findings of most of the studies are promising and researchers concluded that all these lectins show antimicrobial properties. However, many more bioactive compounds mainly lectins in skin mucus of fishes contribute the antimicrobial effect on pathogens. Lectin molecules are carbohydrate binding proteins that are produced by various organisms mainly and can be used as the antimicrobial agents (Coelho et al. 2018). Fish lectins play a key role in their immunity and are present in many tissues and organs such as skin, gills, liver, kidney, intestine and blood of fishes and are well studied (Elumalai et al. 2019). Moreover, these lectins are isolated from fish skin from mucus cells, and the possibilities are there as the utilization of fish mucus and lectins to prevent or treat the pathogenic diseases of fishes.

In many microbes, their resistance to antimicrobial agents makes it difficult to prevent or treat the infections. To overcome these problems, many studies have concentrated on natural bioactive compound from various organisms to treat the microbial diseases. Lectins from many fishes are capable to inhibit or kill the microbe, which act as the natural and potent antimicrobial agents and are able to bind with carbohydrate molecules on the surfaces of microbes (Coelho et al. 2018). This antibacterial effect of these fish lectins occurs when they interact with cell wall components of microbes, which effects in the pore forming of bacteria and also damaging the permeability of the cell. The antifungal activity of these lectins is related to the binding property of lectin to chitin, causing the cessation of fatty acid synthesis in the cell wall of the fungus during its growth or division, which leads to the loss of integrity of the cell wall (Coelho et al. 2018). Some of the lectins are reported with the presence anticancerous and antiviral activities. Lectins are commonly classified according to the carbohydrate binding specificity, cellular location, biological functions, and calcium (Russel et al. 2008). Various studies reported that this lectins show antimicrobial activity and immunomodulatory actions (Ferreira et al. 2011). Moreover, many researchers are investigating on the fish lectins and their inhibition activities or bactericidal properties.

11.1.1 Fish Lectins: An Overview

Lectins consist of a group of protein specifically characterized by carbohydrate-binding ability with the occurrence of carbohydrate recognition domain (CRD) in their base structure (da Silva Lino et al. 2014; Elumalai et al. 2019). These proteins are diverse in nature and found in several organism such as bacteria, virus, yeast, cyanobacteria, plants and animals (da Silva Lino et al. 2014). Lectins are

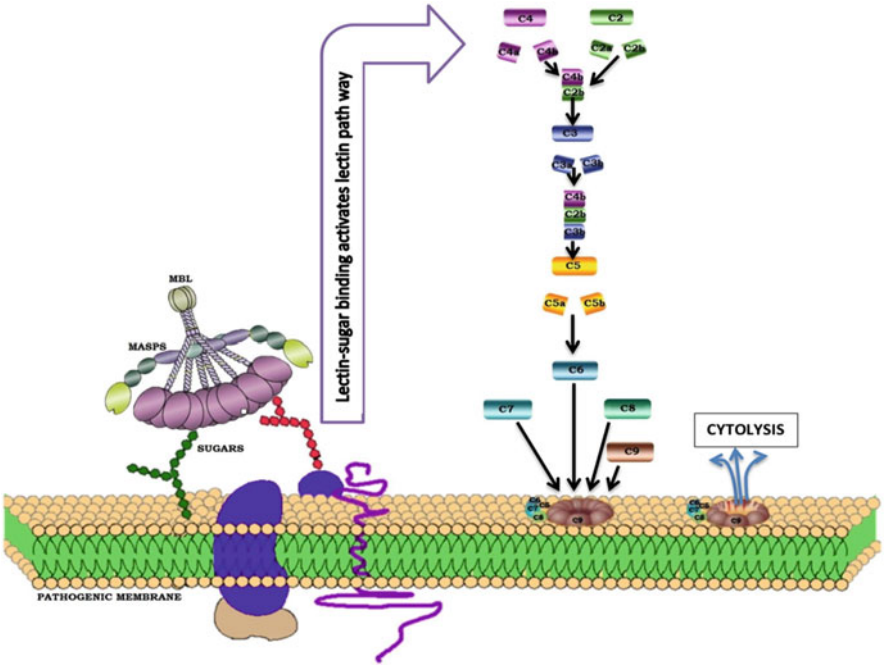

Fig. 11.1 Mechanism of action of lectin on pathogens. (Adapted from Elumalai et al. 2019)

capable of adhering microbial cells and can precipitate polysaccharides and carbohydrate-containing lipids and proteins (Zhang et al. 2009). In the CRD, the lectins bind to the protein by several weak interaction and bond such as ionic attractions, van der Waals forces, hydrophobic and hydrogen bond, and these interaction can make the particular and transient nature of protein–ligand bonds (Fig. 11.1) (da Silva Lino et al. 2014). Lectins show ubiquitous nature and play major role in cellular process like proliferation, agglutination, opsonization, apoptosis, signal transduction and metastasis (Dutta et al. 2005).

The research on fish lectins has enhanced new aspects in fish immunology and lectin biology. Lectins have been isolated from various organs and part of fish such as serum, skin mucus, plasma, egg surface and other components of fish (Dutta et al. 2005). The research on the biological significance is very few, but it plays a major role in morphogenesis, fertilization, poly-spermy block and defence against microorganisms (Dutta et al. 2005). A few number of fish lectins may be associated with intracellular compartments mediating process, mainly trafficking proteins and protein folding by splicing of RNA (Vasta et al. 2011). It is released in the extracellular compartments at two sites: cell surface or as a soluble part of biological fluids (da Silva Lino et al. 2014). In vitro studies of pathogens show the isolation of mucosal lectins from mucus and tissues (Brinchmann et al. 2018). In the mucus, it is found both intracellularly and extracellularly in Golgi apparatus/endoplasmic reticulum (ER) secretory pathway (Brinchmann et al. 2018).

The innate immune responses are the primary defence system of the vertebrates, and several proteins such as lectins and lysozyme have an important part in it. In the case of various bacterial species, lysozymes attack and disturb the cell wall of polysaccharides and kill the microorganism. In the innate immune defence mechanism, mostly events include the identification of microbial objectives for lectins, such as collectins. With the help of carbohydrate expression, these proteins identify the foreign cells and act as opsonins and boost their demolition by complement or phagocytic cells (da Silva Lino et al. 2014). Various fish lectins are supposed to facilitate pathogen identification.

The Atlantic salmon serum consists of the mannose-binding lectins having antimicrobial activity against *Aeromonas salmonicida*, and it has been believed that these lectins have similar properties with the mannose-binding lectin of mammal which play a vital role in innate immunity (Ottinger et al. 1999; da Silva Lino et al. 2014). It has been noticed that the isolation of lectins from the cobia (*Rachycentron canadum*) ovary exhibited the antimicrobial movement contrary to the bacteria such as *Escherichia coli* and no antifungal activities for *Fusarium oxysporum, Rhizoctonia solani, Mycosphaerella arachidicola* and *Coprinus comatus* (Ngai and Ng 2007). According to one study, the lectin isolated from the mucus of catfish (*Silurus asotus*) shows the activity contrary to the pathogenic bacteria such as *A. salmonicida* and suggests that lectins present on the skin surface of the catfish play a major role as a self-defence against the bacterial species (Tsutsui et al. 2011; da Silva Lino et al. 2014). Different patterns of hemagglutinating activity are shown by the lectin segregated from the egg chum salmon (*Oncorhynchus keta*) (Shiina et al. 2002). The lectin isolated from Chinook salmon roe (*Oncorhynchus tshawytscha*) exhibits no agglutination properties or antifungal activity towards various fungal species (Bah et al. 2011; da Silva Lino et al. 2014).

11.1.2 Classification of Fish Lectins

The earlier classification of animal lectins was based on the structural evidence of the protein section responsible for relation with CRD and divided into two categories: S-type and C-type (Drickamer 1988). S-type lectins consist of no disulphide bonds, present intracellularly and extracellularly and identify primarily galactose molecules. C-type lectins consist of large superfamily of membranes and extracellular proteins and share a disulphide-rich Ca^{2+}-binding CRD (Ewart et al. 2001). A large number of new animal lectins were emerging based on the activity and structural information of carbohydrate or CRD specificity. The information on biological function or evolutionary classification is very limited, but new research has been investigating the role and function of lectins in animal with the help of immunological and molecular biological process (da Silva Lino et al. 2014; Ewart et al. 2001). A description of major lectin families associated with fish is as follows.

11.1.2.1 C-Type Lectins

The C-type lectins (CTLs) are remarkable by the requirements of Ca^{2+} and wide specificity for carbohydrates or its binding to mono- and oligosaccharides (Vasta et al. 2011). It is classified into several groups that consist of the proteoglycan core proteins, collectins, selectins, mannose-macrophage receptor and endocytic receptors. Mannose-binding lectin (MBL) can also include in the family of CTLs, and it is a vital component of innate immunity in mammals (da Silva Lino et al. 2014; Vasta et al. 2011). Various types of CTLs having specificity for carbohydrates or CRD have been isolated from various parts such as plasma, tissues and skin mucus of various teleost fish that include carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), fugu (*Takifugu rubripes*), zebrafish and eel (*Anguilla japonica*) (Vasta et al. 2011). It is also identified in other fish species such as Japanese flounder (*Paralichthys olivaceus*), lamprey (*Petromyzon marinus*), grass carp (*Ctenopharyngodon idella*) and venomous fish (*Thalassophryne nattereri*) (da Silva Lino et al. 2014). The CTLs are diverse in functions and associated with several processes including pathogen recognition, endocytosis, cell adhesion, platelet activation, tissue integration and remodelling, phagocytosis, cytotoxic effect, antibacterial and mitogenic activities (da Silva Lino et al. 2014).

11.1.2.2 S-Type Lectins or Galectins

These are galactosyl-binding lectins and designated as thiol-dependent proteins which recognize mainly β-galactosides (Drickamer 1988; Vasta et al. 2011). It requires reducing environment for activity such as binding beta-galactosides and Ca^{2+}-independent activity (da Silva Lino et al. 2014; Vasta et al. 2011). Gelectins are subdivided into three major types, based on its primary structure and polypeptides structural, including the proto-type, chimaera-type and tandem-repeat type, and these lectins are abundant in cycstol (Vasta and Ahmed 2008; da Silva Lino et al. 2014). The biological functions of these lectins are not well known, but they contribute in immune system and development process. The first galectin known as Electrolectin was segregated from the electric eel (*Electrophorus electricus*), specifically from the electric organs, and it has been noticed in teleost family that all galectin types, proto, tandem-repeat and chimera present in it are abundantly found in the mucus, plasma and various tissue of teleost fish and elasmobranch (Vasta et al. 2011). It has been noticed that galectins contribute to various physiological process in animal such as immunity, development of cells, proliferation of malignant cells, apoptosis and morphogenesis and play a major role in the defence system of fish (Elumalai et al. 2019).

11.1.2.3 F-Type Lectins

The F-type lectins or FTLs, also known as fuolectins, consist of a large number of proteins and share distinct sequence F-type motif. It is the latest family of lectins related to fish that recognized and structurally considered in teleosts fish (Vasta et al. 2011; Elumalai et al. 2019). The F-lectins present in the plasma of teleosts act as acute phase reactants and as a response against stress, injury or infection; it quickly increase in the plasma (Elumalai et al. 2019). In mostly teleosts, the FTLs comprise either duplicate such as in striped bass, Zebrafish or quadruplicate such as in steelhead trout with tandemly arranged domains of subunits of diverse sizes and agglutinin holds a single F-type CRD in the European eel (*Anguilla Anguilla*) (Vasta et al. 2011).

11.1.2.4 Pentraxins

It comprises serum amyloid P (SAP) and C-reactive protein (CRP) bounded by non-covalent bond (Bottazzi et al. 2009). C-reactive protein is a prototypical pentraxin, activates complement and differentiates self from non-self and neutralizes bacteria. It reveals lectin-like properties that include the attachment to carbohydrates and its linked structures and requires divalent cation and have a role in biological functions (Bottazzi et al. 2009). It consists of a highly preserved family and has been labelled in various fish species which include carp, common wolffish, snapper, cod, rainbow trout and halibut (Vasta et al. 2011).

11.1.2.5 Calnexin and Calreticulin

Calnexin and calreticulin are proteins that are found in the endoplasmic reticulum and represent a group of intracellular lectins. These lectins work as molecular sensors that examine an N-linked oligosaccharide precursor that identifies a non-reducing-end glucose residue in it, interrelates transiently with glycoproteins and ensures the mechanism of folding but mostly acts to maintain misfolded proteins in the endoplasmic reticulum (da Silva Lino et al. 2014; Vasta et al. 2011). They have a high-affinity site for Ca^{2+}-binding and Zn^{2+}-binding sites inside the globular domain and both sites bind ATP, but no detection of ATPase activity has been observed (Leach et al. 2002). In the eukaryotic cells, they play various biological, physiological and immunological functions which include the oxidative stress responses, lectin binding and the regulation of intracellular calcium homoeostasis. These lectins identified in various animals such as mammals, plants and fish species which include rainbow trout, channel catfish, etc. (da Silva Lino et al. 2014). Kales et al. (2004) isolate calreticulin from rainbow trout and Fuller et al. (2004) identified calnexin in the channel catfish.

11.1.2.6 Rhamnose-Binding Lectins

Rhamnose-binding lectins or RBLs exhibit specific binding affinity and isolated from the eggs of *Oncorhynchus mykiss*, *Silurus asotus*, *Scomberomorous niphonius* and *Salvelinus leucomaenis* (Elumalai et al. 2019; Terada et al. 2007; Tateno et al. 2002). The first member identified and isolated in the RBL family was Sea urchin egg lectin (SUEL) (Tateno 2010). This family has been stated in more than 20 species of fish and mainly situated in ovaries and oocytes and skin mucus (Elumalai et al. 2019). Various RBLs are dispersed in the adult tissues that include thrombocytes, blood leukocytes and spleen, and it is synthesized in oocytes and liver (Vasta et al. 2011).

11.2 Functional Activities of Fish Lectins

Lectins play vital role in various biological functions of animals such as defence system, chemotaxis, endocytosis, phagocytosis, etc. Various lectins have specific function in the system (Table 11.1). In the Atlantic salmon plasma, mannan-binding lectin increases phagocytosis and killing mechanism of *Aeromonas salmonicida* and *Vibrio anguillarum* after binding in a calcium-dependant manner (Ewart et al. 1999; Ottinger et al. 1999; Colquhoun and Sørum 2001). At very low concentrations, Blue gourami lectin promotes phagocytosis of bacteria and also located to cohere strains of *Aeromonas hydrophila*, and it is also identified to be attached with the cell surface of macrophage cell (Russell and Lumsden 2005). The skin lectins that are generated in the skin and established in the mucus layer of the Japanese eel have been found to agglutinate bacteria. *Anguilla japonica* lectin-1 (AJL-1) and AJL-2 has been noticed to agglutinate with *Escherichia coli* and *Streptococcus difficile*, respectively, and the growth of *E. coli* also inhibits by AJL-2 (Russell and Lumsden 2005).

Lectins play a major role in animal's immune system by protecting against diseases, and they act as opsonins, endocytic uptake of pathogens and promoting the phagocytic (Brinchmann et al. 2018). But lectins can also help the pathogens that result in promoting the infections. It has been noticed that in the gills of catfish (*Ictalurus* spp.), mRNA of rhamnose-binding lectin has been achieved to be regulated early after *Flavobacterium columnare* infection which cause columnaris disease, and during the infection, rhamnose-binding lectin was stimulating infection instead of inhibiting it (Sun et al. 2012; Beck et al. 2012). In vulnerable fish, infection is repressed by D-galactose and L-rhamnose, presumed rhamnose-binding lectin ligands. In chum salmon (*Oncorhynchus keta*), phagocytosis activity is promoted by rhamnose-binding lectins after binding to globotriaosylceramide (Gb3) (Beck et al. 2012; Watanabe et al. 2009).

The F-type lectin was expressed and localized in the intestine and liver of sea bass (*Dicentrarchus labrax*), and it increases the phagocytic activity against *E. coli* by the specialized macrophages. In the Atlantic cod, galectin-1 is reported from head

Table 11.1 Immunomodulatory roles of lectins

Lectin family	Lectin	Source	Mode of action	Target	References
F-type lectins	GANL (220 kDa)	Bighead carp	Growth inhibition by agglutination	*Vibrio harveyi*	Pan et al. (2010)
Galectins	AJL-1 (30 kDa) PfGAL9 (35.12 kDa) Congerin LcGal9 Pufflectin	Japanese eel Yellow catfish Conger eel Yellow croaker Puffer fish	Growth inhibition by agglutination Cellular encapsulation Binds parasite	*Streptococcus difficile* *E. coli, A. hydrophila, B. subtilis* *B. negaterium* and *S. aureus* Bacteria Parasitic nematodes *V. alginolyticus* and *A. hydrophila*	Tasumi et al. (2004), Wang et al. (2016), Nakamura et al. (2012), Zhang et al. (2016), Suzuki et al. (2003)
Intelectins	SaIntL (35 kDa)	Catfish	Growth inhibition by agglutination	*Aeromonas salmonicida*	Tsutsui et al. (2011)
Fish-egg lectins	RbFEL (28.2 kDa) Coho egg lectin zFEL KPL (140 kDa) RBL (30 kDa)	Rock bream Coho salmon Zebrafish Skipjack tuna Chinook salmon	Enhances lectin activity on challenged with pathogens Binds bacteria Phagocytosis of microbes by macrophages Haemagglutination Anti-proliferative activity	*Edwardsiella tarda*, *Streptococcus iniae* and red sea bream iridovirus (RSIV) *Aeromonas salmonicida* *Aeromonas hydrophila* Blood type A erythrocytes Human breast cancer MCF-7 cells and hepatoma Hep G2 cells	Kim et al. (2011), Yousif et al. (1995), Wang et al. (2016), Jung et al. (2003), Bah et al. (2011)
C-type lectins	HjCL BGL MBL (80 kDa) SauFBP32 LycCTLR OniL (17 kDa) RcaL MBL	Japanese bullhead shark Pallus Potcha fish Atlantic salmon Yellow croaker Tilapia fish Cobia fish Cobia fish	Blood coagulation Binds bacteria Promotes phagocytosis Activates immune response Exhibits strong cytotoxic effects Binds to bacteria Induces higher IFN-γ production Lowers IL-10 as well as nitrite release Induces production of IL-2 and IL-6 and proliferative responses Mitogenic activity	Erythrocytes (shark blood) *Edwardsiella tarda* *Aeromonas hydrophila* Mice *Vibrio anguillarum* and *A. salmonicida* *E. coli and B. cereus* Mouse splenocytes	Tsutsui et al. (2015), Fock et al. (2001), Absar et al. (2008), Ewart et al. (1999), Ao et al. (2015), da Silva et al. (2012), Coriolano et al. (2012), Ngai and Ng (2007)

kidney leukocytes that had beads of phagocytosed latex, signifying that phagocytosis intensify the secretion of lectin so that it can further intensify phagocytosis activity during infections (Brinchmann et al. 2018).

The pathogens enter in the cells by interacting the host surface with pathogen ligands, and the blocking of this pathway can be a good defence mechanism against the diseases caused by these pathogens. The galectins found in the zebrafish, Drgal3-L1 and Drgal1-L2, have been applicable against the binding of glycoprotein in infectious haematopoietic necrosis virus (IHNV) (Brinchmann et al. 2018). The base of the fin is the site for virus to enter in the host, and in vitro experiment shows that adhering of the virus to fish epithelial cells is prevented by the galectins (Nita-Lazar et al. 2016). Instead of forbidding the virus entry, lectins can also stimulate the uptake of virus by host organism. The C-type lectin found in the black rockfish (*Sebastes schlegelii*) increases the number of infectious spleen and kidney necrosis virus (ISKNV) in the black rockfish (Brinchmann et al. 2018). So, it has been cleared that each lectins have their own specific function in specific animal. They can impose the activity of pathogens as well as stimulate it.

11.3 Therapeutic Potential of Fish Lectins

Protein–carbohydrate interactions are a critical component of the host defence mechanism. Several investigations have been shown that glycobiological interactions play a vital role in a wide range of immunological processes (the interaction of lectins with glycan complex on pathogens and their molecules itself, Fig. 11.1) (Dam and Brewer 2010; Gleeson 2008; Rabinovich et al. 2012). Carbohydrate forms like glycoproteins and glycolipids have been implicated in a variety of pathological and physiological functions, including host–pathogen interaction and cell to cell communication in a variety of cell types; i.e., when it comes to drug development from organic sources, lectins are the most possibilities for the construction and development of inhibition against bacterial diseases (Ogawa et al. 2011; Bonazzi and Cossart 2011; Rabinovich et al. 2012). The biorecognition event is crucial for accurate identification, treatment and healing of diseases (Cheung et al. 2015).

Several lectins have received a lot of interest for their anti-HIV, anti-inflammatory, antinociceptive, anti-tumour and antibacterial properties in biomedicine (Ogawa et al. 2011; Sun et al. 2008). Different physical changes, such as the disease development, impact the cellular protein glycosylation pattern. As a result, any change in the glycan component of a glycoprotein could be indicative of the disease that caused the change. Consequently, the tremendous advances in the ability to exploit, block or activate protein–carbohydrate interactions, lectin and carbohydrate study are at the frontier of science., particularly in medicinal chemistry (Hudak and Bertozzi 2014; Sharon 2008). A vast variety of lectins were found in marine species with probable medical uses in recent decades; for example, lectins can be employed

as surface markers in cancer detection and treatment (Mody et al. 1995; Ghazarian et al. 2011).

11.4 Interaction and Response of Lectins with Other Compounds

The lectins are diversified in nature and can be isolated from a wide range of sources like virus, bacteria, fungi, animals, plants and algae. They show special specificity towards carbohydrate molecules such as mannose, fucose, sialic acid, complex glycans, glycoproteins and galactose/*N*-acetylgalactosamine. The main characteristic of lectins is the identification of carbohydrates and glycoconjugates in various organ in the animal body, and this property can help as biotechnological tool and in the field of diagnosis, pharmacological and therapeutic applications (Coelho et al. 2017). On the surface of host cells, lectins interact with glycan chains to facilitate the infection process, which allows the virus to enter the cell (Van Breedam et al. 2014). Lectins also contribute highly to the bacterial virulence. The bacterial lectin bind with particular carbohydrate moieties of host cells that become a significant factor for adhesion and recognition (Coelho et al. 2017). The lectins associated with animal system play various role in physiological process which include immunomodulation, metastasis of cancer and apoptosis pathways (Kilpatrick 2002; Liu et al. 2012). The separation of various lectins from the animal tissues were found as immunomodulatory, apoptotic agents, anticancer drug targets and antiviral therapy.

Various lectins from different sources show immunomodulatory effects mainly mitogenic activity and induction of T helper cell, and these activities start after binding with glycan targets present on cell surface, which have specific role of lectin receptors (Coelho et al. 2017). After the binding of lectin, the immune reactions induce through intermediaries such as second messengers that originate from the membrane of cell. The immunostimulatory effect is shown by the rhamnose-specific lectin, which is isolated from grass carp fish ovaries. The mitogenic activity on mouse splenocytes was exhibited by mannose-binding lectin isolated from the fish cobia ovary (Ngai et al. 2007). It was reported that mannose-binding lectin behaves as immunomodulatory agent which is differentiated from serum of cobia which is known as *Rachycentron canadum* lectin (RcaL) (Coelho et al. 2017).

Galectins found in the animal play a major role in immunomodulatory processes. In the maturation of thymocyte, regulatory role is regulated by Galectin-1 (Baum et al. 1995). Apoptosis of immature thymocytes is induced by galectin-1 through the activation of p53 pathway, and it inhibits the cell growth (Coelho et al. 2017). It has been intended that galectin-1 stimulates apoptosis by regulating intracellular signals (Yang et al. 1996). The apoptosis process can be prevented by Galectin-3 in which the membrane of mitochondria is protected and inhibits the reactive oxygen species (ROS) production (Rabinovich et al. 2002).

11.5 Antibacterial Activity of Lectins When Combined with Other Antimicrobial Agents

The interaction of lectins with components of the bacterial cell wall such as lipopolysaccharides, teioic acid, peptideoglycans and carbohydrates leads to agglutination or reduction of cell growth in Gram (−ve) and Gram (+ve) bacteria (Bourne et al. 1994). They can also restrict cell development by modifying lowering nutrient absorption, cell permeability, subsequent eruption of extracellular material and pore formation or via interfering with intracellular response-enhancing membrane receptors (Mukherjee et al. 2014). Recognition of a lectin's specificity for certain carbohydrates can aid in preventing subsequent infections by blocking the bacteria's interaction with the host cells (Iordache et al. 2015). Several studies have shown that antibiotics used to treat harmful bacteria can be made more effective by using plant derivatives (de Oliveira et al. 2011). Synergistic interactions between conventional antibiotics and organic products can be an effective technique for combating bacterial hindrance (Cheesman et al. 2017).

Moura et al. (2021) investigated and found that the lectins derived from the heartwood (MuHL), leaves (MuLL) and bark (MuBL) of *Myracrodruon urundeuva* have been anti-staphylococcal, and the same ones linked to cefoxitin and cefotaxime have an interaction effect. Moura et al. (2021)MuBL, MuHL and MuLL had bacteriostatic and bactericidal activities against MRSA clinical isolates and *S. aureus* NCTC 8325 (MBC = 100 g/mL and MIC = 12.5–50 g/mL, respectively). All combinations had a synergistic impact, with MuBL–cefotaxime, MuLL–cefoxitin, MuLL–cefotaxime and MuBL–cefoxitin combinations having the largest MIC reduction of 14-fold against CA-MRSA. The conjunction of MuBL and cefoxitin demonstrated an enhancing effect against 8325 strain. The lectins extracted from the *Schinus trebinthifolia* (SteLL) has a synergism against *S. aureus* NCTC 8325 if combined with ciprofloxacin (de Souza Feitosa Lima et al. 2019).

Procópio et al. (2019) examined the lectin derived from the *Calliandra surinamensis* (CasuL) leaves against aprine mastitis, bovine segregates have anti-staphylococcus properties, CasuL–tetracycline conjunction had a synergistic impact against *S. aureus,* CasuL–ampicillin conjunction had a synergistic effect against *Staphylococcus* sp., with ApuL, a lectin isolated from the *Alpinia purpurata* flowers, was shown that ApuL with oxacillin had a synergistic effect against two MRSA strains, and ApuL with ceftaidime had a synergistic effect against MDR *P. aeruginosa* (Ferreira et al. 2018). *S. aureus* has been the most common cause of mastitis and nosocomial infections in dairy cattle (Procópio et al. 2019; Ventola 2015). Its many virulence determinants, such as biofilm generation, as well as varied protection profiles, such as Vancomycin-resistant *S. aureus* (VRSA) and MRSA, allowed it to adjust to various domain, demonstrating its adaptability in various toxicological conditions (Nickol et al. 2019).

Silva et al. (2019) evaluated the antibacterial activity of lectin derived from *Punica granatum sarcotesta* (PgTeL) bactericidal efficacy against *E. coli* developing-lactamase (CMY, MBL and CTX-M) strains, as well as possible

pharmaceutical interactions. In this study, PgTeL–ceftazidime was observed to have a synergistic impact against four ESBL-positive *E. coli* strains and one MBL-positive *E. coli* extract, PgTeL–carbenicillin against an ESBL-positive extract, PgTeL–ampicillin against an ESBL-positive strains and PgTel–cefuroxime against three ESBL-positive strains. Ceftazidime MICs were reduced 4- to 128-fold, ampicillin MICs were reduced 33-fold, carbenicillin MICs were reduced 16-fold, and cefuroxime MICs were reduced 256- to 1000-fold (Silva et al. 2019). Another study found that using a lectin from *Acinetobacter baumannii* in conjunction with ceftazidime against *E. coli* and *S. aureus* resulted in a considerable boost; in comparison to monotherapy, there was an eight-fold decline in MIC against both infections (Muslim 2015).

ESBL-positive strains have been linked to infections on nearly every continent (Iovleva and Bonomo 2017). In Gram-negative bacteria, -lactamases are resistant to -lactams, and the third-generation cephalosporins have several pathways (Tooke et al. 2019). Carbapenems are among the possibilities for treating MDR bacteria and increase selective pressures on carbapenemase-producing strains' emergence and dissemination (Sengupta et al. 2013). Because antimicrobials have a limited spectrum of action, dosing with lectins has proven to be a viable approach (Khan and Gurav 2018). Santos et al. (2020) recently released two studies that looked into lectin antimicrobial activity and its relationship with medications. In the first study, a lectin obtained from Dioclea violet seed (DVL) has been evaluated, but while it seemed to have no clinical activity if tested lonely (MIC 1024 g/mL), the DVL–gentamicin combo substantially enhanced antibacterial effects, reducing the gentamicin MIC from 32 to 12.7 g/mL against MDR *E. coli* and from 50.8 to 10.1 g/mL against MDR *S. aureus*.

Silva-Angulo et al. (2015) got comparable findings against MDR *S. aureus* using a lectin isolated from *Parkia platycephala* (PPL) seeds. Although PPL had no antibacterial activity (MIC 1024 g/mL), it did lower the gentamicin MIC from 64 to 25.4 g/mL. The MIC values was lowered by 37.5% and 80% against MDR *E. coli* and MDR *S. aureus*, respectively, if lectin derived from the *Canavalia ensiformis* (ConA) leaves was coupled with gentamicin, despite ConA having no antimicrobial effect (MIC 1024 g/mL) when it is used alone (Santos et al. 2021). Gentamicin is an aminoglycoside antibiotic with such a wide range of antimicrobial properties which is used to treating nosocomial infectious diseases caused by staphylococcal and Gram-negative bacteria. It could be used in conjunction with -lactam antibiotics to expand therapeutic coverage (Alhariri et al. 2017; Almeida et al. 2013). Studies have shown that combining aminoglycosides with natural products improves their antibiotic efficacy, making them a promising option for reducing bacterial resistance (Almeida et al. 2013).

The CuS nanoparticles (CuS NPs) which has previously been found to exhibit a broad range of antibacterial activities conjugated with shrimp lectin (Md-Lec). As a result, its MIC towards marine microbes was four times lower than when they were acting alone (Elumalai et al. 2021).

In each and every reports, lectins were found to enhance the therapeutic impact of commercial antimicrobials, resulting in a lower MIC. According to these studies,

when coupled with antibiotics, the lectin might be employed as an enhancer in the treatment of infectious diseases.

11.6 Future Perspectives

The variety of lectins extracted from marine resources, notably fish, has grown in recent decades. Fish lectins have a wide range of structural and functional features, including carbohydrate specificities that are unique and specific. The number of lectins identified, isolated and physiochemically characterized is growing at an exponential rate. Functional features, particularly lectins with downstream effects, and confirmation of their significant in important fish disease are mostly unreachable. In fish, lectins are a component of the innate immunity and play a key role in host defence, especially with the stimulation of the complement pathway. The functional disability of specific lectins likely causes many Infectious diseases in fish due to genetic mutation in lectins. But little is recognized about the biological process of functional properties of lectins in fish immunogenicity, it apart from their affirmation in tissue related to innate immunity, their down- or up-regulation by contagious challenge, and their adhesion to pathogenic organisms.

Furthermore, knowledge on the molecular and regulatory aspects of fish lectins has received low attention when compared to information gathered from mammalian researches. In terms of economic benefits, increased production of disease-resistant fish would be extremely beneficial to the industry. Current genomic and transcriptomic methodologies on fish models are expected to provide comprehensive understanding of their involvement as identification and activator proteins in host defence or regulators of adaptive immunity at the molecular scale in the future.

Conflict of Interest The authors have no conflict of interest to declare.

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Part IV
Functional Role of Fish Lectins

Chapter 12
Antimicrobial and Immunomodulatory Role of Fish Lectins

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Abstract Lectins are proteins that can bind to carbohydrates selectively and reversibly without changing the ligand's covalent structure. Lectins are divided into several families based on their structure, binding specificities, and calcium dependence, such as C-type lectins, I-type lectins, F-type lectins, intelectins, rhamnose binding lectins, galectins, Lily-type lectins, and so on. Innate immunity and disease resistance are known to be aided by lectins such as ficolins, calnexin, galectins, F-type lectins, intelectins, and mannose-binding proteins (MBPs). In fish, skin serves as an essential immunological structure, and lectins have been found in the stomach, eggs, gut, liver, gills, serum, skin, and plasma of several fish species. The capacity of lectins to promote agglutination, suppression of planktonic development, biofilm inhibition or eradication, and/or death of bacteria has been shown to have direct antibacterial action. The interaction of lectins with bacterial cell wall components [such as *N*-acetylglucosamine (NAG), *N*-acetylmuramic acid (NAM), tetrapeptides related to NAM, and lipopolysaccharides] as well as membrane receptors has been credited with growth suppression and death induction. This might cause permeabilisation and the development of holes in the bacterial cell wall and membrane, allowing intracellular material to seep out. Lectins are part of the innate immune system's humoral component and are engaged in the detection of PAMPs that cause agglutination and neutralisation of potentially microbial pathogens or the activation of complement components. Various research findings show that fish lectins play an important role in immunological identification of microbial infections and phagocytosis clearance. The significance of lectins in antimicrobial and immunomodulatory activity in fish is discussed in this review.

Keywords Fish lectins · Antimicrobial activity · Immunomodulatory activity · Galectins · CTL · FTL

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Abbreviations

CRD	Carbohydrate-binding lectin
CTL	C-type lectin
CTLD	C-type lectin-like domain
EPN	Glu-Pro-Asn
Es-Lec	*Etroplus suratensis* lectin
FTL	F-type lectin
GlcNAc	*N*-acetylglucosamine
MBL	Mannose-binding lectin
NAM	*N*-acetylmuramic acid
QPD	Gln-Pro-Asp
RBL	L-rhamnose-binding lectin
STL	S-type lectin
SUEL	Sea urchin egg lectin
XTL	X-type lectin

12.1 Introduction

The name 'lectin' refers to a group of carbohydrate-binding proteins found in viruses, prokaryotes, and eukaryotes that are extensively dispersed (Sharon and Lis 2004). William Boyd and Elizabeth Shapleigh introduced the word from the Latin word, which has the meaning 'to select', in 1954 (Boyd 1970). It etymologically reflects their properties to selectively aggregate blood cell groupings. The research of lectins was first concentrated on plants alone, as the protein was thought to occur in the plant world only. In the study of impacts of plant toxicity in 1888, Stillmark found the primary agglutinin in the crude extract from *Ricinus communis*. Lectins from *Phaseolus vulgaris*, *Phaseolus lunatus*, and *Canavalia ensiformis* are isolated and identified as first plant lectins (Sharon and Lis 2004). The study of glycobiology made a big breakthrough in the late twentieth century since the scientific community recognised the presence of lectin in animal tissues. Stockert et al. (1974) probably found the first mammalian lectin in rabbit liver, and the first serum lectin was identified as the hepatic asialoglycoprotein receptor by Ashwell and Morell (1974) and (Hudgin et al. 1974). Animal lectins have produced major advancements in glycobiology, glycoside decoding, and the development of several fundamental and applied bioscience sectors. In several biological processes including infection and metastasis, the engagement of lectins with carbohydrates is the main event. Besides this crucial function in cell reconnaissance, in numerous areas of study where saccharide specificity is critical, the interaction between lectins and carbohydrates has been investigated. These proteins are important tools for the investigation of simple or complex carbohydrates, either in solution or on the cell

surface as well as for the characterisation of cells since they are capable of discriminating between countless complicated forms.

Lectins have been categorised by particular carbohydrates they have recognised, but categorisation is also based on structural information, given the rising numbers of lectins. At least one non-catalytic carbohydrate-recognising domain (CRD) is present in these proteins, which can bind sugars reversibly and with great specificity without changing their covalent structure (Kilpatrick 2002). The spatial configuration of both amino acids that form the CRD and adjacent amino acids determines the uniqueness of the carbohydrate-binding site; in addition, metal ions may aid in the proper placement of the carbohydrate for binding. Lectin CRDs can attach to simple or complex sugar structures via hydrogen bonds, hydrophobic interactions, as well as van der Waals forces (Lemme 2013). Animal lectins are classified into several families based on their CRD sequence motifs and cation requirements, including C-type lectins (CTLs), galectins (formerly S-type lectins), X-type lectins, P-type lectins, F-type lectins, rhamnose-binding lectins, I-type lectins, and pentraxins (Gabius 1997).

The development and maintenance of highly specialised mutual connections in organism–microbe complexes has already proved that protein–carbohydrate interaction forms the foundation of processes that provide mediatory signal-related activities, cell communication, and self-identification. Mutual advantage (symbiosis or commensalism) in this regard depends on maintaining a strictly controlled equilibrium, whereas colonisation of tissues that are advantageous to microbe fitness might result in loss (pathogenesis) of host fitness unless host defensive responses remove pentraxins from foreignness (Ballarin et al. 2013). Microheterogeneity derived from numerous copies of the lectin genes, allelic variations, or gene modifiers enhance molecular diversity and the ability to recognise the product.

Innate and adaptive immunological responses are part of the immune system of vertebrates. There are several proteins, such as lysozyme and lectins in the innate immune system. The lysozyme attacks and disturbs polysaccharides of various bacterial species in the cell wall killing microorganisms (Parisien et al. 2008). The identification of microbial targets for lectins, for example collectins, is the major event in innate immune defence (Holmskov et al. 1994). These proteins detect foreign cells as non-self by the surface expressed carbohydrates that function as opsonins and promote death by complementary and/or phagocytic cells. As phylum, fish have been used to help us understand how the immune system is evolving, as despite significant differences, the immune sys of fish physiologically resembles that of higher vertebrates. Like mammals, an innate and adaptive fish immune system includes both cell-mediated protection and humoral components. The intrinsic characteristics of disease resistance are key variables. Fish adaptive response is often delayed, but is necessary for long-term immunity.

12.2 Fish Lectins

In both the cartilaginous and the bony fishes, CTLs, FTLs, galectins, and pentraxins were discovered (Elumalai et al. 2019). In addition, in the already known fish, genomes, selectins, and other lectin genes were identified. The isolated fish serum, skin mucus, and other tissue were mostly lectin families in mammals, including CTLs, XTLs, and galectins. In addition, certain fish-unique lectin families like RBL were discovered in eggs and embryos as well as in the serum (Ogawa et al. 2011). Although the functional importance of lectins in innate immune responses is quite well established in mammals, lectins and other innate immune effectors may play a critical role in fishes' learned immunological responses. Several research studies have been conducted on the role of lectins in the immune system of fish, and it is thought that several fish lectins influence pathogen detection in the immune system. Lectins can exercise opsonic activity or improve breathing and phagocytic cell bactericides (Yang et al. 2011). Humour and host membrane-related lectins are important recognition molecules that can enable beneficial associations with colonial bacteria or can activate innate and adaptive responses against harmful pathogens. Fish lectins also act as mediators for other activities, such as agglutination, fertilising, immobilisation and supplementary opsonisation, and pathogen kills (Sharon and Lis 1972). In intracellular intermediation activities, such as splicing of RNA to fold proteins and trafficking proteins, certain fish lectins may also be found. Several research studies on the involvement of lectins in the immune system of fish have been published in recent decades.

12.3 Classification of Lectins

Lectins are structurally varied molecules with a vast number of families reflecting the structural diversity inherent in these proteins. The structures of lectins are crucial for defining the properties of glycan classes present in a variety of species, and animal lectins are now classified into distinct groups based on common evolutionary origin and/or structural fold similarity. We aim to outline the key animal lectins that also occur in fish in this section, stressing their main features and activities. Based on the structural information of the protein component responsible for carbohydrate interaction, CRD, the first categorisation of animal lectins separated these proteins into two categories: S-type and C-type. Since then, other lectin groups have developed, based primarily on CRD structural data. Lectins have been linked to a direct first-line defence against infections, cell trafficking, immunological modulation, and autoimmunity prevention.

12.3.1 S-Type Lectins (STLs or Galectins)

Galectins are a class of carbohydrate-binding proteins that have a Ca^{2+}-independent affinity for β-galactosidases sugar and share a 130-amino-acid-residue-long conserved sequence motif inside its CRD. They are thiol-dependent proteins with intracellular and extracellular localisation that primarily recognise β-galactosides (Barondes et al. 1994). However, for certain lectins in this group, the necessity for thiol reducing agents is unclear; the name alectin has been applied to this group because of similar features such as the capacity to bind beta-galactosides and Ca^{2+}-independent action. The proto-type (galectins 1, 2, 5, 7, 10, 11, 13, and 14), chimaera-type (galectin 3), and tandem-repeat type (galectins 4, 6, 8, 9, and 12) are the three forms of galectins found in the cytosol (Kasai and Hirabayashi 1996). Galectin-4 has two CRD linked by a link peptide, whereas galectin-3 has one CRD connected to two domains, resulting in multimers (Lobsanov and Rini 1997). Galectins are thought to play a role in a variety of physiological processes, including cell formation, differentiation, morphogenesis, immunity, apoptosis, and malignant cell metastasis, among others. Teichberg et al. (1975) isolated electrolectin from the electric eel's (*Electrophorus electricus*) electric organs, making it the first galectin to be identified and characterised (Teichberg et al. 1975). Numerous galectins have been identified in various fish species, including the Japanese eel (Tasumi et al. 2002), zebrafish (Vasta et al. 2004), windowpane flounder (*Lophopsetta maculate*) (Kamiya and Shimizu 1980), conger eel (*Conger myriaster*) (Shiomi et al. 1989), and channel catfish (*Ictalurus punctatus*) (Zhang et al. 2012).

12.3.2 C-Type Lectins (CTLs)

CTLs are a type of lectin found in fish that binds to mono and oligosaccharides in a Ca^{2+}-dependent manner. CTLs are divided into numerous categories, including collectins, proteoglycan core proteins, and selectins that are either directly or indirectly engaged in immune activity. C-type lectins (CTL) and proteins with C-type lectin-like domains (CTLD) are members of the C-type superfamily. Calcium ions are required for CTL to bind to carbohydrates. The calcium domain is substantially conserved in all members of the family, but the kinds of carbohydrates recognised and the CRD are generally different. In vertebrates, the Glu-Pro-Asn (EPN) or Gln-Pro-Asp (QPD) motifs are important sugar-binding residues. Cell adhesion, tissue integration and remodelling, platelet activation, complement activation, pathogen identification, endocytosis, phagocytosis, cytotoxic impact, mitogenic and antibacterial activity, as well as specific antibody formation are all processes that C-type lectins are involved in (Cummings and McEver 2009). Selectins and collectins usually feature multi-domain structures and one or more highly conserved CRDs, which have a distinctive mixed α and β topology and consist of 115–130 amino acid residues. MBL can alternatively be categorised as

part of the C-type lectin superfamily based on CTL or CTLD characterisation. The mannose-binding lectin (MBL) is a well-studied component of innate immunity in animals. MBL is calcium-dependent to sugars with hydroxyl groups on carbon-3 and carbon-4 oriented in the equatorial plane of the pyranose ring; it has affinity for mannose, fucose, and *N*-acetyl glucosamine (GlcNAc). The protein functions as an opsonin, increasing phagocytosis of foreign material it has bound and activating the lectin route of complement activation via MBL-related serine proteases (Ip et al. 2009). Rainbow trout (*Oncorhynchus mykiss*) (Jensen et al. 1997), Japanese eel (*A. japonica*) (Tasumi et al. 2002), catfish (*Silurus asotus*) (Tsutsui et al. 2011a), fugu (*Takifugu rubripes*) (Zelensky and Gready 2004), common carp (*Cyprinus carpio*) (Savan et al. 2004), have all been found to have CTLs with different carbohydrate specificities (Table 12.1).

12.3.3 I-Type Lectins

I-type lectins are lectins that recognise particular sialylated glycoconjugates and facilitate cell–cell interactions. They are structurally related to the immunoglobulin superfamily. I-type lectins that bind sialic acid (Siglecs) are a structurally different subclass of I-type lectins. They are membrane proteins that are expressed primarily on the plasma membrane. CD83 and cell adhesion molecule L1 are structurally distinct from Siglecs, although they appear to recognise sialic acids as well (Crocker and Varki 2001).

12.3.4 Pentraxins

Pentraxins are made up of several subunits ranging in size from 20 to 25 kDa, each containing one CRD. These lectins bind to saccharides on bacterial cell surfaces in a Ca^{2+}-dependent manner and are seen in serum as acute phase proteins, indicating that they play a function in the defence system. They can also be membrane-associated (CRP), with a preference for phosphorylcholine, or extracellular matrix-associated, with a preference for phosphoethanolamine (Mantovani et al. 2008). Pentraxins were shown to have opsonin activity in the snapper *Pagrus auratus*, indicating that they have a role in the host defence fish (Cook et al. 2005). In reaction to stress, damage, or infection, several pentraxins act as acute phase proteins, rapidly increasing their plasma concentration up to 1000-fold or more and influencing immunological responses. Pentraxins are a structurally related family of proteins found in a variety of fish species, including common carp (*Cyprinus carpio*) (Cartwright 2004), snapper (*Pagrus auratus*) (Cook et al. 2005), wolffish (*Anarhichas lupus*) (Lund and Olafsen 1998), Atlantic halibut (*Hippoglossus hippoglossus*) (Magnadóttir et al. 2019), and rainbow trout (Jensen et al. 1995). However, there are changes in the structure of native CRPs found in channel catfish

Table 12.1 Classification of lectins and their biological roles

Type of lectin	Biological function	Feature	References
S-type lectin	Inflammatory reactions; cell growth regulation and apoptosis; development, differentiation, morphogenesis, tumour metastasis, apoptosis	β-Galactosides for binding; Ca^{2+}-independent action	Fukumori et al. (2007)
C-type lectin	Cell adhesion (selectins): Lymphocyte homing (L-selectin); leukocyte trafficking to sites of inflammation (E- and P-selectins); innate immunity (collectins); promote phagocytosis, complement activation (MBL); apoptosis and cell growth regulation	Activity that is dependent on Ca^{2+}, with a conserved Ca^{2+} binding location	Arnold et al. (2006), Ourth et al. (2008) and Kerrigan and Brown (2009)
I-type lectins	Cell–cell interactions; cell routing; immune and neurological systems	Affinity for sialic acid and structural similarities to the immunoglobulin superfamily	Varki and Angata (2006)
Pentraxins	Detection of abnormal or invasive cell glycosylation (endocytosis or initiation of opsonisation or complement activation)	Ca^{2+}-dependent acute phase protein found in serum	Kilpatrick (2002) and Magnadottir (2010)
F-type lectins	Innate immunity and molecular recognition	Ca^{2+} independent, non-glycosylated affinity for L-fucose	Salerno et al. (2009)
Calnexin	Endoplasmic reticulum folding process and misfolded protein retention; stress-induced apoptosis	Intracellular lectin	Tamaoki et al. (2004) and Williams (2006)
L-rhamnose binding lectins	Regulation of carbohydrate metabolism, fertilisation, cell proliferation, cytotoxicity, opsonisation, stimulation of respiratory bursts, and microbicidal activity	Two to three homologous CRD in tandem of about 95–100 amino acid residues bind L-rhamnose	Terada et al. (2007) and Watanabe et al. (2009)

compared to the human counterpart. Rainbow trout and Atlantic salmon serum have been found to contain SAP-like proteins (*Salmo salar L.*) (Lund and Olafsen 1999). When the expression and function of pentraxins in tongue sole (*Cynoglossus semilaevis*) were elucidated, a pentraxin called CsPTX1 was found to suppress in vitro growth of *Pseudomonas fluorescens* (Wang and Zhang 2016).

12.3.5 F-Type Lectins

F-type lectins are non-glycosylated, Ca^{2+}-independent, and selective for L-fucose. Honda et al. (2000) called this protein category fucolectin (Honda et al. 2000). Among the known lectins, the ell fucolectin has a structure that is unique. Many biological interactions are mediated by the presence of terminal L-fucose as a non-reducing terminal residue on different glycoproteins and glycolipids. During inflammation, the expression of fucose-containing antigens has been found to skyrocket. Fuc-TVII, a fucosyl-transferase, is required for efficient neutrophil and T-cell recruitment to inflammatory areas, as well as lymphocyte trafficking to secondary lymphoid organs, in animals (Smithson et al. 2001). The presence of seven types of clones was discovered using northern blot analysis, three of which were from the liver and coded for similar but different proteins with 180 amino acid residues. Teleost F-lectins are found in the plasma of healthy fishes and act as acute phase reactants, rapidly raising their plasma concentration in response to stress, injury, or infection. In Japanese eel agglutinin (AAA), structures with single CRD and tandem CRDs of FTLs have been described, whereas European eel agglutinin (AAA) has a single F-type CRD (Gupta and Gupta 2012). Modern teleosts such as Nile tilapia (*O. niloticus*) (Argayosa and Lee 2009), Japanese sea perch (*Lateolabrax japonicus*) (Qiu et al. 2011), and striped bass (*Morone saxatilis*) (Bianchet et al. 2010) have tandem-repeated forms of F-type lectins.

12.3.6 Calnexin and Calreticulin

Calnexin and calreticulin are related intracellular lectins, proteins of the endoplasmic reticulum that bind transiently with glycoproteins and may or may not participate in the folding mechanism, but most likely serve to maintain misfolded proteins in the endoplasmic reticulum. Have a lectin site on the folding glycoprotein $Glc_1Man_9GlcNAc_2$ that detects an early oligosaccharide processing step. Calnexin and calreticulin contain a high-affinity Ca^{2+}-binding site and bind Zn^{2+} at locations inside the globular domain; both bind ATP, but there is no evidence of ATPase activity. Calreticulin has a variety of physiological and immunological roles in the eukaryotic cell, including intracellular calcium homoeostasis control, lectin binding, and oxidative stress responses in mammals (Zhang et al. 2014).

12.3.7 L-Rhamnose-Binding Lectins (RBL)

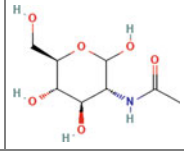
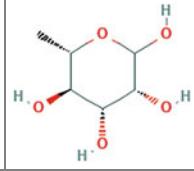
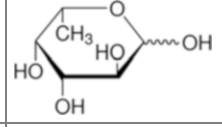
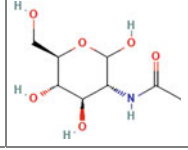
RBLs, or rhamnose-binding lectins, are lectins found in fish and invertebrates that have a special affinity for L-rhamnose. The RBL family's first member, sea urchin (*Tripneustes ventricosus*) egg lectin (SUEL), was identified and sequenced (Tateno

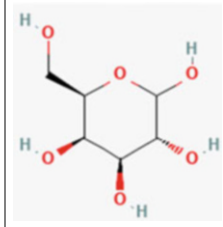
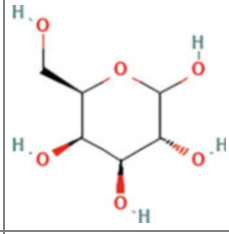
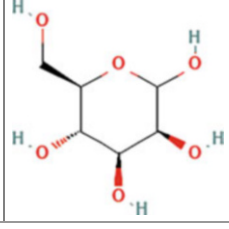
2010). RBLs have been found in more than 20 fish species, mostly in oocytes and ovaries, as well as skin mucus, and a new family of rhamnose-binding lectins (RBLs) has been discovered in steelhead trout eggs, Spanish mackerel, and catfish eggs. RBL group of lectins was proposed based on their carbohydrate binding selectivity and molecular structure, which comprises two or three homologous CRD in tandem of around 95–100 amino acid residues with a distinctive topology and a set of conserved motifs. The domain design, hemagglutinating activity for human erythrocytes, and carbohydrate specificity are used to divide RBL into five subgroups. It is also thought to have a role in inflammatory reactions by acting as agents of cell identification and trafficking to inflammatory areas, as well as activating the inflammatory cascade via modulating cytokine production. The ability of RBLs to detect and bind lipopolysaccharide and lipoteichoic acid, as well as agglutinate both Gram-positive and Gram-negative bacteria, suggested a function in egg protection against infectious challenge (Cammarata et al. 2014). Bacteria that were exposed to RBL were more likely to be phagocytosed by peritoneal macrophages. RBL's opsonic activity and bacterial agglutination capacity support the theory that RBL facilitates not only agglutination and immobilisation of potentially pathogenic microorganisms but also promotes their phagocytosis and clearance from circulation, thereby playing a significant role in host defence against infectious challenge. The presence of RBLs in skin mucus backed up their postulated immunological function(s) (Cammarata et al. 2014). The glycosphingolipid globotriasylceramide (Gb3), which is abundant in membrane lipid rafts, has been discovered as a possible natural ligand of fish RBL (Cammarata et al. 2014) (Table 12.2).

12.4 Distribution of Lectins Within Fish

Serum, gills, surface mucus, egg surfaces, and components have all been found to contain lectins, but the skin of various animal species, particularly fish, is thought to be a significant source of novel and unreported lectins. The diversity of lectin expression and tissue location in fish, implying that they have different functions in innate immunity from the moment oocytes are released and fertilised, through larval, juvenile, and adult stages. The first fish lectin was discovered in the exterior mucus of the *Ophidiidae* family (*Genypterus blacodes*) (Oda et al. 1984). Many lectins have been isolated from the stomach, intestine, liver, gills, eggs, skin, mucus, serum, and plasma of various fishes since then, which will be discussed accordingly (Ng et al. 2015).

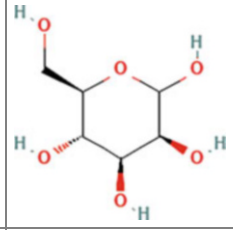
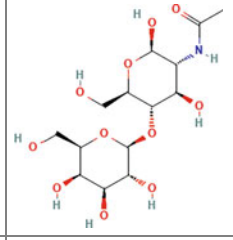
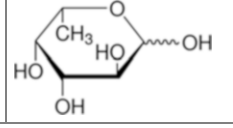
Table 12.2 Fish lectins, their sources, and binding specificity of lectins (all structures of carbohydrate obtained from https://pubchem.ncbi.nlm.nih.gov/)

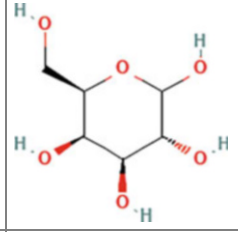
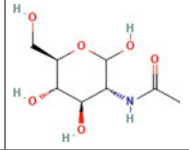
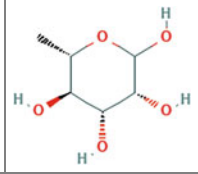
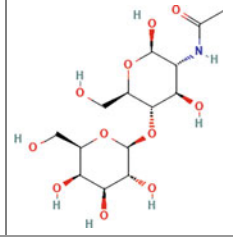
Lectin type	Fish species	Lectin	Binding specificity	Structure	References
C-type lectin	Fugu (*Takifugu rubripes*)	Kallik lectin	*N*-acetyl glucosamine		Tsutsui et al. (2015b)
Fish egg lectin	Rock bream (*Oplegnathus fasciatus)*	RbFEL	Rhamnose		Kim et al. (2011)
Fucolectin	European eel (*Anguilla anguilla*)	AAA	Fucose		Bianchet et al. (2002)
C-type lectin	Indian catfish (*Clarias batrachus*)	CBL	*N*-acetyl glucosamine/ galactose		Singha et al. (2008)

					
C-type lectin	Japanese eel (*Anguilla japonica*)	eCL-1, eCL-2	Galactose		Mistry et al. (2001)
C-type lectin	Tilapia fish (*Oreochromis niloticus*)	OniL	Mannose		da Silva et al. (2012)

(continued)

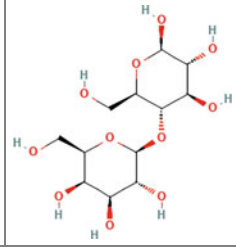
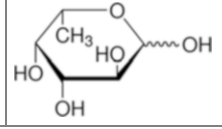
Table 12.2 (continued)

Lectin type	Fish species	Lectin	Binding specificity	Structure	References
Lily-type lectin	Rock bream (*Oplegnathus fasciatus*)	OfLTL-2 and 3	Mannose		Lee et al. (2016)
Galectin	Yellow croaker (*Larimichthys crocea*)	LcGal9	*N*-acteyllactosamine		Zhang et al. (2016)
C-type lectin	Atlantic salmon (*Salmo salar*)	SauFBP32	Fucose		Soanes et al. (2004)

C-type lectin	Japanese bullhead shark (*Heterodontus japonicus*)	HjCL	Mannose		Tsutsui et al. (2015a)
C-type lectin	Pallus (*Trichogaster trichopterus*)	BGL	*N*-acetyl glucosamine		Fock et al. (2001)
RBL	Channel catfish (*Ictalurus punctatus)*	IpRBL	Rhamnose		Thongda et al. (2014)
Galectin	Japanese eel (*Anguilla japonica*)	AJL-1	*N*-acetyllactosamine		Tasumi et al. (2004)

(continued)

Table 12.2 (continued)

Lectin type	Fish species	Lectin	Binding specificity	Structure	References
C-type lectin	Japanese eel (*Anguilla japonica*)	AJL-2	Lactose		Tasumi et al. (2004)
F-type lectin	Bighead carp (*Aristichthys nobilis*)	GANL	Fucose		Pan et al. (2010)

12.4.1 Skin Mucus

Skin mucus is regarded as the primary defence structure since it is in frequent touch with water that may carry a variety of pathogens. As a result, it contains immunological molecules called lectins. Agglutinins, lysozymes, lysins, and immunoglobulins are found in fish skin mucus, in addition to lectins. The epidermal mucus of the pink cusk-eel produced the first fish lectin, which was isolated from the epidermal mucus (*G. blacodes*) (Oda et al. 1984). HjCL, a CTL related to snake venom lectins, was isolated from the skin of a Japanese bullhead shark (*Heterodontus japonicus*) (Tsutsui et al. 2015a). Tsutsui et al. (2011a) discovered intelectins in catfish skin for the first time, and the lectin demonstrated mannose binding activity in the presence of calcium (Tsutsui et al. 2011a). RT-PCR experiments indicated that the lectin gene is also expressed in the gills and kidneys of catfish, despite the fact that these intelectins were first discovered in the skin of the fish. FHL, a homodimeric lectin, was found in the flat head's skin mucus (*Platycephalus indicus*) (Tsutsui et al. 2011b). Tsutsui et al. (2015b) recently published the genome sequencing of skin mucus CTLs from 16 *Anguilla* species and 3 subspecies (Tsutsui et al. 2015b). The lectins AJL-1 and AJL-2 from Japanese eel, congerin from conger eel, pufflectin from pufferfish (*T. rubripes*), and PFL-1 and -2 from pony fish (*Leiognathus nuchalis*) were discovered in skin mucus (Okamoto et al. 2005).

12.4.2 Serum

The CTL, BGL from blue gourami (*Trichogaster trichopterus*) that was selectively bound to *N*-acetyl glucosamine, enhanced the innate immune response against *A. hydrophila*, a common fish disease-causing bacteria (Fock et al. 2001). A multimeric mannose binding lectin constituted of 17,000 M_r subunits was separated from the blood serum of Atlantic salmon (Ewart et al. 1999). RcaL with 19.2 kDa was isolated from cobia (*Rachycentron canadum*), which did not display any cytotoxic activity, but the serum lectin was able to instil higher interferon gamma and nitric oxide production in splenocyte cultures (Coriolano et al. 2012a). The serum of fugu contains a *N*-acetyl glucosamine binding kalliklectin, which is a homologue of kalliklectin and has similar characteristics to mammalian plasma kallikreins and coagulation factor XI (Tsutsui et al. 2015b). From the serum of Indian catfish, Dutta et al. (2005) identified a calcium and pH-dependent galactose binding lectin with a molecular mass of roughly 200 kDa (Dutta et al. 2005). This lectin had a-methyl galactose and sialoglycoprotein specificity. When sea bass (*Dicentrarchus labrax L.*) were exposed to bacterial infection and confinement stress, their F-lectin, DlFBL, was regulated (Parisi et al. 2015). A soluble lectin with a molecular mass of 68 kDa and activity dependent on Ca^{2+}, Mn^{2+}, or Mg^{2+} was discovered in the serum of spotted snakeheads *(Channa punctatus*) (Manihar and

Das 1990). In the serum of European eels, a fucolectin AAA was discovered, which plays a role in pathogen detection.

12.4.3 Gills

Mistry et al. (2001) discovered two new C-type galactose-binding lectins in freshwater Japanese eels, eCL-1 and eCL-2 (Mistry et al. 2001). Another family of lectins, FTL, GANL, with an assumed molecular mass of 220 kDa, was identified from bighead carp gills (Pan et al. 2010). Six RBLs (IpRBL1a, IpRBL1b, IpRBL1c, IpRBL3a, IpRBL3b, and IpRBL5a) were discovered in the gills of channel catfish with one to three CRDs containing the YGR and DPC conserved patterns (Thongda et al. 2014).

12.4.4 Fish Eggs

Lectins are abundant in fish eggs. The RbFEL, a fish-egg lectin, was discovered in rock bream (*Oplegnathus fasciatus*) and shown to have six conserved residues in the fish egg lectin family, as well as all cysteine sites in each domain (Kim et al. 2011). In coho salmon *(O. kisutch)*, galactophilic fish-egg lectin was found to bind to *Aeromonas salmonicida*, the causative agent of furunculosis, and zFEL from zebrafish, a maternal factor increasing macrophage phagocytic activity against Gram-positive and Gram-negative bacteria. In chinook salmon, a rhamnose-binding roe lectin with a molecular mass of 30 kDa and high stability at 70 °C and pH levels ranging from 4 to 11 was discovered (Yousif et al. 1995; Wang et al. 2016).

12.5 Antimicrobial Action of Lectins

Lectins are without a doubt the most versatile protein family employed in biological and medicinal research. The ability of lectins to recognise cell surface carbohydrates has far-reaching consequences in biorecognition technologies aimed at studying the structure and function of complex carbohydrates, as well as mapping changes in cell surface throughout physiological and pathological processes. The carbohydrate–lectin interaction is also responsible for the immunomodulatory, anti-inflammatory, anticancer, hypotensive, insecticidal, antiviral, antifungal, and antibacterial effects of lectins (Paiva et al. 2012). Bacterial cell surfaces are covered in a variety of carbohydrates, including lipopolysaccharides (glycoconjugates found in Gram-negative bacteria, composed of a lipid, an oligosaccharide core, and an O-antigen polysaccharide chain) and peptidoglycans [present on Gram-positive and Gram-negative bacteria's cell walls, formed by β(1 → 4), linked *N*-acetylmuramic

(NAM) and *N*-acetylglucosamine (NAG) as alternate residues NAM is linked with tetrapeptides] (Vollmer et al. 2008). The interaction of lectins with bacterial cell surfaces can cause agglutination and cell growth suppression. Lectins can generally agglutinate bacterial cells at doses lower than those required to limit growth; agglutination causes bacteria to become immobilised, which may make it easier for the lectin molecules or other antibiotic agents to work on a large number of cells (Costa et al. 2010). The binding of lectins to glycoconjugates on the bacterial surface may limit growth by a variety of processes, including changes in cell permeability, which reduces nutrient absorption, and/or interactions with membrane receptors, which activate intracellular reactions. Lectins are a diverse collection of proteins, and even those with similar sequences can differ in carbohydrate-binding affinity, and biological actions of a single lectin on various microorganisms may change due to the variation of carbohydrate organisation on microbial cell surfaces (Karnchanatat 2012).

Antibacterial animal lectins are more commonly present in tissues that have direct contact with the external environment, such as the skin, respiratory system, and digestive tract, and act directly or indirectly against pathogens via several methods. The recognition of a pathogen by animal lectins may lead to rapid clearance of the microorganism via opsonisation, phagocytosis, and oxidative burst by immune system cells. Animal lectins are classified as C-type and S-Lac-type lectins, with calcium-dependent and calcium-independent carbohydrate-binding abilities, respectively. Collectins and selectins are C-type lectins that have more than one carbohydrate recognition domain. Selectins are membrane-bound receptors, whereas collectins are water-soluble. Soluble C-type lectins act directly on pathogens by a variety of methods, including cross-linking between the lectin and the bacterial surface, which induces immobility and makes phagocytosis easier. In vertebrates, these lectins may activate the complement system, whereas invertebrates may activate the melanisation system. When linked to a pathogen, Dectin-2 promotes the production of pro-inflammatory cytokines such interleukin (IL)-1β, IL-6, and TNF-α (Yabe and Saijo 2016). Bulb-type mannose-specific lectins from the fish *Cynoglos sussemilaevis* have antibacterial efficacy against Gram-negative and Gram-positive bacteria, according to Sun et al. (2016). These lectins are likewise Ca^{2+}-dependent and have a non-selective bactericidal activity, allowing them to decrease bacterial infection under in vivo circumstances. Both vertebrate and invertebrate species produce S-Lac-type lectins, also known as galectins, which are selective for β-galactoside (β-Gal) and *N*-acetyl-D-lactosamine (LacNAC) residues. The majority of them are bivalent proteins that work against bacteria by attaching to glycan epitopes such as LacNAc moieties found in lipopolysaccharides, lipooligosaccharides, and capsular oligosaccharides. Galectins-3 is generated by resident alveolar macrophages in response to *Streptococcus pneumoniae* infection, according to Farnworth et al. (2008), and activate neutrophils, which phagocyte bacteria and release cytotoxic mediators (Farnworth et al. 2008).

Three L-rhamnose-binding lectins (STL1, STL2, and STL3) isolated from *Oncothynchus mykiss* eggs were shown to impede *E. coli* and *B. subtilis* cell growth and agglutinate cells. STL3 had the best impact on *E. coli*, preventing 50% of growth

when used at 60 μg/mL, compared to STL1 (20%) and STL2 (15%) when administered at 250 μg/mL. At 250 μg/mL, STL1, STL2, and STL3 inhibited *B. subtilis* growth by 40%, 30%, and 30%, respectively (Tateno et al. 2002). Because all three lectins may bind O-antigen moieties in bacterial cells, the authors speculate that lipopolysaccharides and lipoteichoic acids are involved in these actions. Only Gram-negative bacteria (*Aeromonas hydrophila, Alcaligenes faecalis, Klebsiella edwardsii,* and *Vibrio metschnikovii*) were able to agglutinate by galactose-specific lectin from *Clarias gariepinus* skin mucus, while Gram-negative and Gram-positive strains were inhibited in growth (*Bacillus polymxa, B. cereus, A. hydrophila, V. metschnikovii, P. aeruginosa, Klebsiella* sp., and *Enterococcus faecalis*) (Olayemi et al. 2015).

Etroplus suratensis (Es-Lec), a C-type lectin from pearl spot serum, possesses yeast agglutination activity against *Saccharomyces cerevisiae* and the capacity to agglutinate human erythrocytes. The antibacterial activity of pure Es-Lec against Gram-negative *Vibrio parahaemolyticus* and *Aeromonas hydrophila* was wide. Purified Es-antibiofilm Lec's capability against Gram-negative bacteria showed disruption of biofilm architecture at a concentration of 50 μg/mL, as well as antiviral and anticancer activity. Es-Lec has a role in the immunological response to pearl spot and provides insight into the pathogen-associated pattern recognition mechanism that activates the complement lectin pathway (Rubeena et al. 2019).

Xue et al. (2013) categorised a serum lectin isolated from lamprey (*Lampetra japonica*) as an intelectin. The intelectin was a new type of extracellular animal lectin that included glycan-binding receptors that bind to glycan epitopes on pathogen surfaces. It also displayed agglutinating properties against *Candida albicans,* a yeast, and Xue et al. (2013) proposed that intelectin plays a crucial role in lamprey innate defence against yeast based on these findings (Xue et al. 2013).

A tetrameric lectin was discovered from the ovaries of cobia, and it showed antibacterial action against *E. coli* at a concentration of 250 μg/mL, with 50% inhibition. The biological purpose of *Ctenopharyngodon idella* MBL (CiMBL) is to start the complement system's lectin pathway. In the presence of Ca^{2+}, recombinant *Ctenopharyngodon idella* MBL (rCiMBL) binds to D-mannose, D-galactose, D-glucose, *N*-acetyl-D-glucosamine (GlcNAc), lipopolysaccharide (LPS), peptidoglycan (PGN), and Agar, where Gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*) and Gram-negative are agglutinated. Furthermore, rCiMBL improves grass carp survivability post bacterial infection (Liu and Dang 2020).

Collectin 11 (CL-11, also known as collectin kidney-1), a fellow member of the vertebrate C-type lectin superfamily, is a pattern recognition protein of the lectin complement system that plays a crucial role in innate immunity. The Nile tilapia protein rCL-11 had a substantial antibacterial impact on *S. agalactiae* and *A. hydrophila*. The Nile tilapia CL-11 protein is an acute-phase protein. Hwang discovered that recombinant human CL-11 could bind to *S. pneumonia* and activate complement in a calcium-dependent way. In eukaryotic systems, rCsCL-11 (a lectin from half-smooth tongue sole *Cynoglossus semilaevis*) has an excellent antibacterial efficacy against both Gram-negative and Gram-positive bacteria (Huang et al. 2019).

Tambaqui serum lectin (ComaSeL), obtained from Amazonian fish *tambaqui Colossoma macropomum*, which was discovered due to its hemagglutinating activity, was employed in antibacterial activity tests against harmful bacteria in freshwater fish. It showed antibacterial action against Gram-negative bacteria by recognising the carbohydrates D-galactose, 1-*O*-methyl-D-galactopyranoside, and D-fucose. Its activity differed significantly between summer and winter ($p < 0.05$, Tukey test), validating previous findings that tambaqui is more sensitive to disease caused by bacteria and fungi during the winter (Marques et al. 2016).

rFc-hsL, a recombinant CTL from Chinese shrimp (*Fenneropenaeus chinensis*), bound Gram-positive bacteria (*Bacillus cereus, Bacillus megaterium, Bacillus thuringiensis, Micrococcus luteus*, and *Staphylococcus aureus*) as well as Gram-negative bacteria (*E. coli* and *Klebsiella pneumoniae*). Ec-CTL, a lectin isolated from orange spotted grouper *(Epinephelus coioides)*, was found to include a CTL with antifungal activity. It bound to and aggregated *Saccharomyces cerevisiae* in a Ca^{2+}-dependent manner, and when challenged with *S. cerevisiae*, lectin expression was upregulated (Runsaeng et al. 2017).

Ellis (2001) revealed in an early review that viral infection causes the production of interferon, which can activate antiviral defences in adjacent cells. Recently, lectins have proven to be useful in the creation of antiviral medications to treat a variety of viral infections, particularly the human immunodeficiency virus type 1 (HIV-1). Lectins attach to mannose sugars, a critical component of the HIV envelope's gp120, and prevent it from changing conformation, keeping it in an inactive, non-usable condition. Since this success, a number of antiviral therapeutic medicines based on lectins (e.g. griffithsin and scytovirin) have been proposed as novel effective antiviral treatments (Ellis 2001).

Recombinant galectins-1 have the potential to neutralise the lymphocystis disease virus (LCDV), decrease cytopathy in infected cells, and reduce inflammation in LCDV-infected cells. Furthermore, when LCDV and galectin-1 were administered, the expression of TNF-a and mx (an antiviral protein) in the gill and head kidney, as well as IL-1β in the head kidney, were considerably reduced. These findings revealed that galectin-1 was responsible for LCDV pathogenicity reduction and antiviral action (Liu et al. 2013).

Although several lectins' actions in marine organism defence systems are recognised, the possibility of using them to other antimicrobial mechanisms has yet to be investigated. More research on lectin therapeutic effects and toxicity, including molecular mechanisms of action, structure–function relationships, and clinical trials, could aid researchers in their investigation. It is also interesting evaluating if lectins have synergistic effects with existing antimicrobial drugs, which could not only add to a better understanding of antimicrobial processes, but could also have therapeutic and drug design implications (Table 12.3).

Table 12.3 Lectin sources and their antimicrobial activity

Lectin source	Activity against Gram-positive bacteria	Activity against Gram-negative bacteria	Activity against fungi	Reference
Clarias gariepinus	*Bacillus cereus, Bacillus polymyxa, Enterococcus faecalis*	*Aeromonas hydrophila, Alcaligenes faecalis, Klebsiella edwardsii, Pseudomonas aeruginosa, Vibrio metschnikovii*		Olayemi et al. (2015)
Oncothynchus mykiss	*Bacillus subtilis*	*Escherichia coli*		Tateno et al. (2002)
Etroplus suratensis		*Vibrio parahaemolyticus, Aeromonas hydrophila*	*Saccharomyces cerevisiae*	Rubeena et al. (2019)
Lampetra Japonica			*Candida albicans*	Xue et al. (2013)
Rachycentron canadum		*Escherichia coli*		Ngai and Ng (2007)
Ctenophoryngodon idealla	*Staphylococcus aureus, Micrococcus leteus*			Liu and Dang (2020)
Fenneropenaeus chinensis	*Bacillus cereus Bacillus megaterium Bacillus thuringiensis Micrococcus luteus Staphylococcus aureus*	*Escherichia coli Klebsiella pneumonia*		Runsaeng et al. (2017)
Epinephelus coioides			*Saccharomyces cerevisiae*	Wei et al. (2010)

12.6 Immunomodulatory Effects of Fish Lectins

Bacteria, virus, fungus, and parasites are recognised and agglutinated by lectins from several fish families. Because of the acute phase response and the large range of microbial species detected, lectins clearly provide an advantage that allows infections to be eliminated quickly before the other immune repertoire is activated. The ability of lectins to bind, agglutinate, and opsonise potential microbial pathogens, as well as their ability to activate complement, suggests that they have not only recognition but also effector capabilities. Various studies have been conducted to determine the involvement of lectins in the activation of innate and adaptive host responses to potential pathogens. According to Vasta et al. (2011), lectins are related

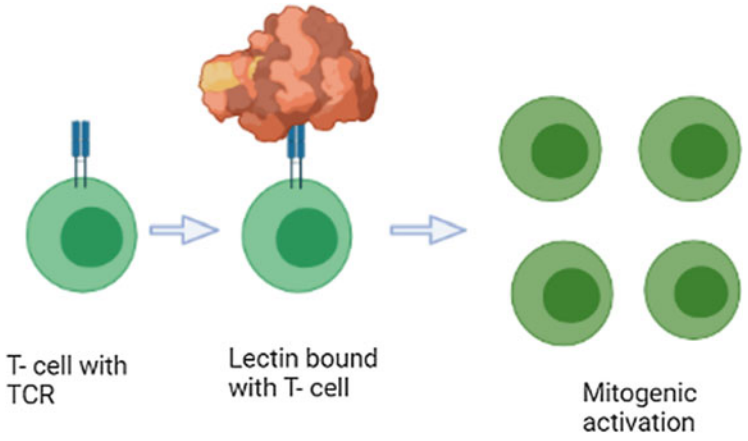


Fig. 12.1 Lectins induced mitogenic activation of T cells

to PAMPs, which lectins recognise via reversible binding through PRRs, resulting in the activation of the lectin pathway of complement activation (Vasta et al. 2011). Lectins' CRD aids in the detection and binding of glycoproteins, glycolipids, and other sugar residues on cell and pathogen surfaces. As a result, they provoke an immunological response by agglutination, immobilisation, and opsonisation of prospective pathogens, as well as complement pathway activation. When rock breams were challenged with pathogenic strains of *Edwardsiella tarda*, *S. iniae*, and red sea bream (*Pagrus major*) iridovirus (RSIV), the expression of fish-egg lectin, RbFEL, was up-regulated, suggesting its role in the innate immune response (Kim et al. 2011). Another galactophilic fish-egg lectin found in coho salmon could bind to *A. salmonicida* but did not decrease bacterial growth, implying that lectins have functions other than disease defence (Yousif et al. 1995). The phagocytosis of microorganisms by macrophages was enhanced by zFEL from zebrafish eggs, and microinjection of zFEL into embryos conferred resistance to *A. hydrophila* (Wang and Zhang 2016). A T-antigen binding lectin (CTL) was isolated from a snakehead murrel (*Channa striata*) in an early investigation, and it was found to have a key role in haemagglutination (Manihar et al. 1990). By agglutination, the FTL GANL proved successful in suppressing *Vibrio harveyi* growth (Pan et al. 2010). Guardiola et al. (2015) described the role of fucose-binding lectin in preventing heavy metal exposure in fish (Guardiola et al. 2015). When Japanese eels were moved from fresh water to seawater, their expression of eCL-1 and eCL-2 dropped dramatically. Because fish are continually exposed to a variety of dangerous microorganisms found in the aquatic environment, the mucus lectins in their skin serve as a crucial barrier. Although active *A. salmonicida* intelectins were found in catfish skin, lectin gene expression was not stimulated by in vivo bacterial assault (Tsutsui et al. 2011a) (Fig. 12.1).

When the purified lectin was coupled with luminarin, the innate immune response to *A. hydrophila* in blue gourami (*Trichogaster trichopterus*) was enhanced, and

bacterium absorption by macrophages enhanced (β-1,3-D-glucan) (Fock et al. 2001). Lectins extracted from Atlantic salmon blood serum attach to mannose residues on the surface of the pathogens *Vibrio anguillarum*, the causative agents of cold water vibriosis, and *A. salmonicida*, revealing the immunological relevance of lectins in Atlantic salmon (Ewart et al. 1999). The galactose-binding lectin identified from Indian catfish binds to a variety of Gram-negative bacteria and affects *Aeromonas* sp. pathogenicity and viability. The galectin could also agglutinate chicken, rat, rabbit, mouse, and human RBCs, as well as trigger the production of IL-1-like cytokines from Indian catfish head kidney macrophages (Dutta et al. 2005). Congerin, a galectin found in the abdominal cavity of conger eels, is involved in the cellular encapsulation of parasitic nematodes, according to Nakamura et al. (2012). LcGal9, a galectin identified from large yellow croaker, was found to be able to agglutinate *Vibrio alginolyticus* and *Acinetobacter hydrophila* (Nakamura et al. 2012).

When tested against human breast cancer MCF-7 cells and hepatoma HepG2 cells, a rhamnose binding roe lectin derived from chinook salmon demonstrated specific anti-proliferative activity and also promoted the formation of nitric oxide in mouse peritoneal macrophages (Bah et al. 2011). The lectin KPL, isolated from skipjack tuna (*K. pelamis*), agglutinated human blood type A erythrocytes with specificity (Jung et al. 2003). HjCL, a CTL isolated from the skin of the Japanese bullhead shark (*H. japonicus*), binds several sugars and causes blood coagulation (Tsutsui et al. 2015a). Lee et al. (2016) discovered that the lily-type lectins OfLTL-2 and 3 are expressed in fully developed immune tissues in rock bream and that these lectins are implicated in the viral infection's second phase (Lee et al. 2016).

A D-galactose-binding lectin from *Musca domestica* pupae was found to have mitogenic action. In vitro, the lectin increased the proliferation of mouse splenocytes, with peak activity at a dosage of 20 μg/mL (Cao et al. 2009). The immunostimulatory action of a rhamnose-specific lectin isolated from the ovaries of the grass carp fish (*Ctenopharyngodon idellus*) was demonstrated in mouse splenocytes and peritoneal exudates cells (Lam and Ng 2002). In treated splenocytes, grass carp lectin stimulated the production of IL-2 and IFNγ. A lectin was also extracted from grass carp roe, and it demonstrated mitogenic action on murine splenocytes and increased the phagocytic activity of sea bream macrophages, comparable to lectin from grass carp ovaries. At 14 μM, a mannose-binding lectin from the ovaries of a cobia fish (*Rachycentron canadum*) was found to have mitogenic activity in mouse splenocytes (Ngai and Ng 2007). *Rachycentron canadum* lectin (RcaL), a mannose-binding lectin isolated from cobia serum, has also been reported as an immunomodulatory agent. Splenocytes from mice in vitro treated with RcaL were tested for mitogenic response and cytokine production. RcaL was found to have a high proliferation index in treated cells and to induce significant amounts of IL-2 and IL-6 production, suggesting that it could be a mitogenic agent (Coriolano et al. 2012b). RcaL generated a Th 1 response in cultured mouse splenocytes by producing IFNα- and NO without causing cytotoxicity. OniL, a mannose-specific lectin derived from the serum of *Oreochromis niloticus* (Nile tilapia fish), induced a Th1 response in mouse splenocytes in vitro. OniL also induced high amounts of

IFNα and low levels of IL-10 and nitrite formation without causing cytotoxicity (da Silva et al. 2012).

Galectin-1 has been found to play a regulatory role in thymocyte maturation by interacting with *O*-glycans on the surface of immature cortical thymocytes (Baum et al. 1995). Galectin-1 also suppressed cell proliferation and promoted death in immature thymocytes by activating the p53 pathway (Perillo et al. 1997), as shown in human leukaemia T cells (Novelli et al. 1999). It is thought that galectin-1 caused apoptosis through regulating intracellular signals such as AP-1 transcription factor activation, Bcl-2 downregulation, and caspase activation. On human leukaemia T cells, galectin-3 acted as a mitogenic and antiapoptotic agent, with apoptosis induced by Fas receptor ligation and staurosporine.

Galectin-3's significant sequence similarity to the antiapoptotic protein family Bcl-2, as well as the existence of the NWGR motif, which is highly conserved in Bcl-2, suggests that it can affect the Bcl-2 pathway and suppress apoptosis. Galectin-3 also protects the mitochondrial membrane and inhibits the generation of reactive oxygen species, preventing apoptosis. Furthermore, through a G-protein route, galectin3 works as a chemotactic agent for monocytes and macrophages (Yang et al. 1996) (Table 12.4).

Apart from their involvement in innate defence, lectins have a number of additional features that can be used in transplant and transfusion medicine. Some of them can also be used to identify stem cell surface markers, specific erythrocyte antigens, and lymphocyte activation. Ahmed et al. (2004) discovered and characterised three galectins that have been shown to play critical functions in embryogenesis (Ahmed et al. 2004). Several lectins have been shown to exhibit immunomodulatory and mitogenic properties. Furthermore, lectin attachment to the target cell surface may trigger an immunological response by releasing mediators such as second messengers from the cell surface. Fungal lectins have been found to be powerful immune response modulators in studies. Lv et al. (2016) revealed that lectins from various sources were found in a recent investigation: (1) act as immunomodulators and biomarkers in vivo and in vitro, (2) aid in mitotic induction, and (3) have a role in infection and inflammation (Lv et al. 2016).

12.7 Conclusion

During the last few decades, the number of lectins isolated from marine resources, particularly fish, has grown. Fish lectins have a wide range of structural and functional features, including carbohydrate specificities that are unique and specific. Lectins are a component of the innate immune system in fish, and they play an important role in innate immunity, particularly in the activation of the complement pathway. The functional impairment of specific lectins caused by lectin gene mutations is likely the cause of species susceptibility or resistance to many infectious fish diseases, but little is known about the molecular mechanisms of functional aspects of lectins in fish immunity, aside from their expression in tissues or organs relevant to

Table 12.4 Fish lectins, their sources, and immunomodulatory effects of lectins

Lectin type	Lectin	Source of lectin	Immunomodulatory effect	Reference
C-type lectins	HjCL	Japanese bullhead shark	Blood coagulation	Tsutsui et al. (2015a)
	BGL	*Trichogaster trichopterus*	Binds bacteria, activates immune response	Fock et al. (2001)
	OniL	Tilapia fish	Binds to bacteria, induces higher IFNγ-c production	Coriolano et al. (2012b)
	RcaL	Cobia fish	Lowers IL-10 as well as nitrite release	Ngai and Ng (2007)
	MBL	Cobia fish	Induces production of IL-2 and IL-6 and proliferative responses mitogenic activity	Ngai and Ng (2007)
Galectins	AJL-1	Japanese eel	Growth inhibition by agglutination	Tasumi et al. (2002)
	Congerin	Conger eel	Cellular encapsulation	Nakamura et al. (2012)
	LcGal9	Yellow croaker	Growth inhibition by agglutination	Zhang et al. (2016)
F-type lectin	GANL	Bighead carp	Growth inhibition by agglutination	Pan et al. (2010)
Fish egg lectin	RbFEL	Rock bream	Enhances lectin activity on challenged with pathogens	Kim et al. (2011)
	zFEL	Zebrafish	Binds bacteria, phagocytosis of microbes by macrophages	Wang et al. (2016)
	KPL	Skipjack tuna	Haemagglutination	Jung et al. (2003)
	RBL	Chinook salmon	Anti-proliferative activity	Bah et al. (2011)

immune functions and their down- or up-regulation by infectious challengers. Furthermore, when compared to knowledge gained from mammalian investigations, information on the molecular and regulatory features of fish lectins has received less attention. In terms of economic gains, higher production of fish with enhanced disease resistance would be of tremendous interest to the industry. Current genomic and transcriptomic methodologies on fish models are expected to provide comprehensive understanding about their involvement as recognition and effector molecules in innate immunity or regulators of adaptive immune responses at the molecular level in the near future.

Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 13
Role of Lectin in Biofilm Inhibition, Haemagglutination, Endocytosis and Phagocytosis

P. Nisha, Manuel Thomas, and T. K. Neelima

Abstract Lectin embodies an exclusive group of proteins with cosmopolitan distribution in nature, which by selection distinguishes and binds to the carbohydrates reversely and with the glyco conjugates by their specific binding sites. The usage of lectin in vitro or in vivo experiments in a broad range of applications in biotechnology, which includes cell and molecular biology, immunological, pharmacological, purposes and in the area of medicine, clinical and drug delivery systems, is well established. Studies on fish lectins are propelled recently to unravel its role which mediates recognition of the presence of the immune system of fishes with an important role, mainly in innate immune responses. Moreover, these fish lectins also have pivotal roles in the fertilization, embryogenesis and morphogenesis. These lectins are in ranges of various molecular weights, glycosylation and its subunit numbers, binding selectivity of sugars/carbohydrates, and amino acid sequence; also their biological effects include antibacterial, anticancer, immunoregulatory and developmental functions. With the evident of genome and transcriptome studies, the full lectin repertoire will be generated with individual participation in the defensive mechanisms, they directly act as the site of recognition also as an effective factors involved in an innate immunity, but they do not directly act as the adaptive immunological reactions, they play a role in defence activities. However, observations on biological effects/roles of each lectin or its isoforms are the need of the hour to engender novel activity spectrum and applications.

Keywords Biofilm · Fish lectin · Lectin · Haemagglutination · Endocytosis and phagocytosis

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13.1 Introduction

William C. Boyd and Elizabeth Shapleigh in 1954 introduced the name 'lectin' (Latin word *lego-* 'chosen'). Lectins are proteins able to adhere with carbohydrates, glycoproteins, glycolipids and proteoglycans. Lectins are a novel molecules present in the nature and found mainly in microbes like bacteria, virus, yeast and cyanobacteria (Xu et al. 2012), plants and also in animals (Nunes et al. 2012). Lectins are grouped in various families based on their specificity to the sugar binding domains, calcium requirements and structural folds (Sharon and Lis 2004). Lectins also have a function in cellular recognition, and numerous biological activities in cells (Brudner et al. 2013) also regulate binding and attachment of microbes including bacteria, viruses and fungi to their own target sites. Lectin molecules are the glycoproteins which are able to bind to carbohydrate such as galactose, lactose, mannose, N-acetyl glucosamine, fucose, rhamnose, etc., and they have a respective specificity (Nilsson 2007). These molecules are with one or more carbohydrate recognition domain (CRD), are binds with specificity and reversibility to the sugars on the surfaces of pathogenic organisms, these lectins are also found to present in several bacteria, some of cyanobacterial sp., virus, fungi, plants and in animals (Veelders et al. 2010). Lectins are involved in various biological functions such as phagocytosis, cell adhesions, innate immunity and complement activations. Lectins are involved in various biological functions such as phagocytosis, cell adhesions, innate immunity and complement activations and are classified into various groups, based on their structure, specific sites of binding, and the Ca^{2+} ion dependency, such as C-type, F-type, intelectins, galectins, rhamnose-binding lectins, I-type and Lily-type lectins (Ogawa et al. 2011). The marine lectins, C-Type lectins or CTL, Ca^{2+} ions binding dependencies to mono and to oligosaccharides, which are again classified into collectins, proteoglycans core proteins, selectins (Vasta and Ahmed 2008).

Lectins having several uses/functions in animals like, regulations in the cell adherence, blood protein levels and glycoprotein synthesis, intracellular and extracellular glycoprotein binding, etc. Lectin has a fundamental role in immune system and is involved in pattern recognition and defensive action against pathogens in vertebrates including fishes (Arasu et al. 2013). Immunal lectins have a considerable role in inflammatory and also autoreactive processes (Maverakis et al. 2015). In the surface of mammalian liver cells, lectins act as a receptor for the recognition of galactose residues and remove glycoprotein from the circulatory system. The plant seed growth decreases in higher concentration of lectins, which convey a role of lectins in the process of plant germination and survival processes of seeds. Lectins are the molecules able to adhere on glycoproteins on the surface portion of parasitic cells, and some of the lectins found in few plant species have been able to distinguish non-carbohydrate ligands which are naturally found to be hydrophobic, include auxins, adenine, indole acetic acid and cytokinin, also water-soluble porphyrins, where many of these molecules act as phytohormones (Komath et al. 2006). Lectins like PHA or concanavalin in leguminous plants can be used as the major model systems for studying the detailed molecular levels in protein recognition of

carbohydrates, and crystalline structures of the lectins in leguminous species revealed the detailed atomic interactions between proteins and carbohydrates.

In microorganisms like fungi, bacteria, virus, protozoa, etc., there is the presence of the lectins, i.e., glycan-binding proteins. Microbial lectins are enumerated depending on the capability to induce or aggregate hemagglutination of RBC cells and conglomerate or stimulate the ability of the erythrocytic cells in hemagglutination phenomenon. Various haemagglutinations are reported in microbes in early 1950, the first hemagglutination in influenza virus was reported as first case, and interpreted by Alfred Gottschalk and Don Wiley and team studied crystallized structure of influenza hemagglutinin in 1981 (Nizet et al. 2017). In some glycoproteins of hepatitis C virus which are able to attach the lectins of C-types to initiate infection on the host liver cell surface (Bartenschlager and Sparacio 2007). Surface lectins like hemagglutinin and adhesins bind to tissue-specific glycans to prevent the body clearance by an innate immunity and pathogenic microbes (Soto and Hultgren 1999).

13.2 Fish Lectins

In fishes, lectins are categorized into various classes like C-type lectins, F-type lectins, lily-type, ricin-type, galactins, rhamnose-binding lectins, and 6x-β-propeller/tectonin-type lectins (Ogawa et al. 2011). Every year, various studies have been performed in fish lectins, getting more structural and functional characteristics (Ogawa et al. 2011). The lectins from some shrimps take part in an innate immunity by the activation of the encapsulation, melanization, prophenoloxidase and the promotion of phagocytosis (Luo et al. 2006). Various studies on characterization and diversity of lectins which lead to an elaboration of explanation to the protein which have non-catalytic carbohydrate recognition domain (CRD) and are an efficient lectins, they are not able to involve in cell agglutination, like membrane bounded proteins, and they have a recognition site on carbohydrate (Ewart et al. 2001).

Fish lectins have a cornucopia of functions mainly immuno-relevant ones like agglutination, pathogen recognition, complement activation, opsonization and phagocytosis and take part in some other functions like RNA splicing, molecular trafficking, folding of protein, and cell proliferation control (Ng et al. 2015). Fish lectins which are found in biological fluids like mucosa and blood as intracellularly and extracellularly. In fishes, a plethora of lectins were separated from the mucosal surfaces with immunological functions and have a role in defence mechanisms in mucosa. Skin, eyelids, gills, noses, mouth, gastrointestinal tract and the urinary bladder of fishes are covered by the surfaces of mucosa, same to mammals. The body surfaces of fishes are always exposed to aquatic environments where pathogens and abiotic factors are present, mucosal surfaces act against these factors as immunological defence systems by mucosal surfaces. Omics Technologies are established about the knowledge which related on the mucosal molecules found in fish skin

(Brinchmann 2016) and the teleost surfaces on the mucosa (Salinas and Magadan 2017) even though only limited information is known about the molecular interaction of mucosal surfaces with bacteria. Also limited knowledge is available on the interaction mechanism between teleost mucosae and yeasts. More studies are to be needed to understand the functions of mucosal surfaces against commensal microbes and pathogenic microbes. In gnotobiotic zebrafish (*Danio rerio*), the normal functions of mucosal cells in some of the functions like nutrient uptake, immunological responses and renewal of epithelial are affected crucially by the microbiota of the gut (Rawls et al. 2004). The mucosal lectins from fish tissues and mucus are subjected to recombinant expression studies with the pathogens as in vitro and also studied histological and binding properties of lectins on to pathogens studies and antibodies against lectins were explicated and distinguished, these lectins were very close to the pathogens in the histological specimens histological and in the binding capabilities (Brinchmann et al. 2018) (Table 13.1).

13.3 Role of Lectins in Biofilm Inhibition

The formation of Biofilm is a multistage process includes the adhesion by the microbes with the secretion and binding of the extracellular matrix, which is composed of polymeric substances like polysaccharides, humic substances, proteins, etc and some of the other molecules which involved in communication in between the cells (Flemming and Wingender 2010). The lectins are able to bind with pathogens and are capable of growth inhibition. Unfortunately, study on lectin assays cannot distinguish between killing and growth inhibition and the result is generally termed as 'antibacterial' (Brinchmann et al. 2018).

Mm-Lec is a lectin molecule with immunological properties, which was isolated from speckled shrimp, *Metapenaeus monoceros,* also has haemolymph and antimicrobial activity against some of the gram positive *Staphylococcus aureus* and *Enterococcus faecalis* and Gram-negative microbes, *Vibrio parahaemolyticus* and *Aeromonas hydrophila* and *Vibrio* the MmLec showed peculiar antibiofilm properties (Preetham et al. 2019). The disturbances in the quarum sensing signalling can interference in the pathogenicity of microbes, and these antimicrobial mechanisms which is not able to lethal effects, also known as anti-virulence therapies, involves disturbing quarum sensing mechanism and formation of biofilm (Silva et al. 2016). A CTL lectin from a Pacific white coloured shrimp *Litopenaeus vannamei* known as Lv CTL3 shows the activation in immune response role against *Vibrio parahaemolyticus* and other viral and bacterial pathogens in shrimps (Li et al. 2014). The B Type mannose-specific lectin molecules isolated from a *Cynoglossus semilaevis* has the survival rate on *Vibrio harveyi* and are pathogenic in a kidney and in spleen (Sun et al. 2016). C-type AJL2 lectins from the Japanese eel incubated with *Escherichia coli* strain showed growth inhibition as per spectrometric assays (Tasumi et al. 2002). The purified lectin Es-Lec from a Pearl Spot (*Etroplus suratensis*) was the antibiofilm activity on biofilm forming *A hydrophila* and

Table 13.1 Various types of lectins from fishes (Ogawa et al. 2011)

Organism	Lectins/lectin family	Binding specificity	Reference
Catfish	D-lactose/galectin		
Arius thalassinus	SAL/Rhamnose-binding lectins	L-Rhamnose	Hosono et al. (1999)
Silurus asotus	Salnt L/Intelectin	D-mannose	Tsutsui et al. (2011a, b)
Steelhead trout, *Oncorhynchus mykiss*	STL-1-3/Rhamnose-binding lectins	L-Rhamnose	Tateno et al. (2001)
Spanish mackerel, *Scomberomorus niphonius*	SML/Rhamnose-binding lectins	L-Rhamnose	Terada et al. (2007)
Sweet fish (ayu), *Plecoglossus altivelis*	SFL/Rhamnose-binding lectins	L-Rhamnose	Watanabe et al. (2008)
White spotted charr, *Salvelinus leucomaenis*	WCL 1,3/Rhamnose-binding lectins	L-Rhamnose	Tateno et al. (2002)
Chum salmon, *Oncorhynchus keta*	CSL-1-3/Rhamnose-binding lectins	L-Rhamnose	Shiina et al. (2002)
Far-East dace, *Tribolodon brandtii*	TBL-1-3/Rhamnose-binding lectins	L-Rhamnose	Jimbo et al. (2007)
Polyfish, *Leiognathus nuchalis*	PFL-1,2/Rhamnose-binding lectins	L-Rhamnose	Okamoto et al. (2005)
Zebrafish, *Danio rerio*	DrgalL1–L3/galectin	LacNAc	Ahmed et al. (2004)
Shishamo smelt, *Osmerus lanceolatus*	OLL/Rhamnose-binding lectins	L-Rhamnose	Hosono et al. (1992)
	OLABL	D-galactose	Hosono et al. (2005)
Windowpane flounder, *Lophopsetta maculate*	Galectin	D-galactose	Kamiya and Shimizu (1980)
Electric eel, *Electrophorus electricus*	Electrolectin/galectin	Lactose	Levi and Teichberg (1981)
European eel, *Anguilla anguilla*	AAA	F-type	Honda et al. (2000)
Conger eel, *Conger myriaster*	Congerin/lactose	Galectin	Muramoto and Kamiya (1992)
	Congerin II/lactose	Galectin	Muramoto and Kamiya (1992)
	conCL-s/mannose	C-type	Tsutsui et al. (2007)
Japanese eel, *Anguilla japonica*	AJL-1/galectin	Beta-Galactoside	Tasumi et al. (2002)
	AJL-2/C-type	Lactose	Tasumi et al. (2004)
	eCL-1/eCL-2/C-type	Lactose	Mistry et al. (2001)
Japanese sea perch, *Lateolabrax japonicus*	Jsp FL/F-type	Fucose	Qiu et al. (2011)
Sea bass, *Dicentrarchus labrax*	DlFBL/F-type	Fucose	Salerno et al. (2009)
Striped bass, *Morone saxatilis*	F-type	Fucose	Odom and Vasta (2006)
Scorpionfish, *Scorpaena plumieri*	Plumieribetin/lily type	D-mannose	de Santana et al. (2009)
Pufferfish, *Fugu rubripes*	Pufflectin/lily type	D-mannose	Tsutsui et al. (2003)
Carp, *Cyprinus carpio*	Carp FEL/6xBeta-propeller/Tectonin	GlcNAc	Galliano et al. (2003)

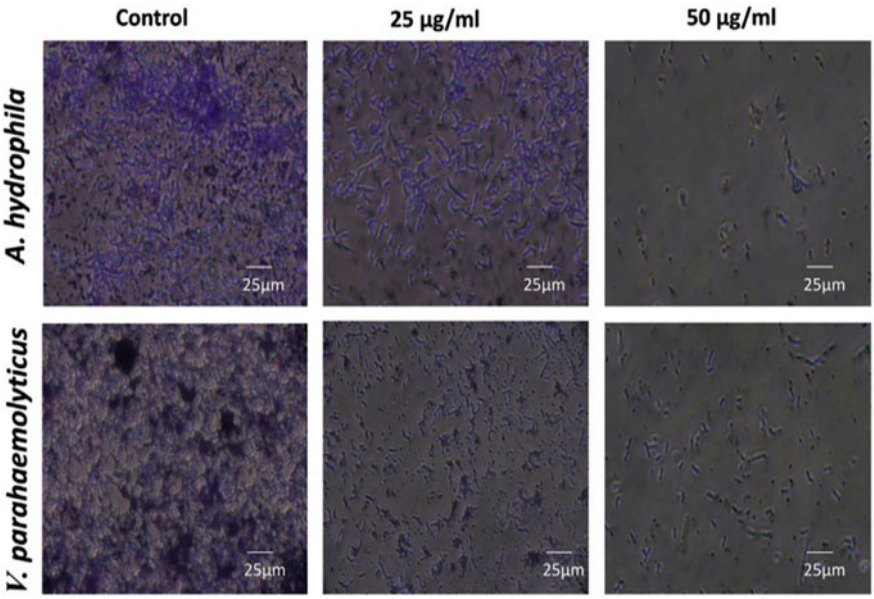

Fig. 13.1 Antibiofilm effect of Es-Lec on biofilm inhibition against *A. hydrophila* and *V. parahemolyticus* demonstrated with crystal violet stain (Rubeena et al. 2019)

V. parahemolyticus. The antibiofilm activity of these Es-Lec (Fig. 13.1) which was increased with increased dose of lectin and were very effective agents against biofilm formation of *A. hydrophila* than *V. parahemolyticus* (Rubeena et al. 2019).

A tetrameric mannose specific lectin is reported, found in the ovaries of Cobia, *Rachycentron canadum* showed an inhibitory zone against *E. coli* on agar. In agar diffusion test, evaluation of antibacterial activity is conducted (Ngai and Ng 2007). Some of lectin molecules with a N terminal carbohydrate-binding domain, Natterin is a Jacalins like lectins has bound with mannose and C-Terminal aerolysin with toxin domain, sublimated from the skin mucosa from Atlantic cod (Rajan et al. 2017) and Oriental stinging catfish (*Plotosus lineatus*) (Tamura et al. 2011) having capacity to lyse pathogens. In vivo effects of fish mannose-binding lectins on MBL from a Nile Tilapia were a role in the defence mechanism against *Streptococcus agalactiae and Aeromonas hydrophila* also was reported (Mu et al. 2017). A variety of mannose binding lectins are able to alter the gene and the protein expression by microbes such as bacteria, virus and fungi, indicating that they are capable for protection of host and may increase amount of lectin which offers protection for host and decrease the infection (Brinchmann et al. 2018). The Channel catfish, *Ictalurus punctatus* with 12 galectin genes and its expression in mucosal tissues and the results showed that it has a changes like tissue type and pathogen type specific on Gram-negative bacterial pathogens (Zhou et al. 2016).

The interactive relation between the surface receptors of the host and the ligands of pathogens gives the easy entry of the pathogens in the host cells. When this

interaction blocked, which serves as a defence mechanism against the entry or penetration of pathogens and the colonization on the surfaces. Galectins, from zebrafish, Drag L1-L2 and Drag L3-L1, are capable of binding with the infectious virus haematopoietic necrosis, known as IHNV glycoprotein. In vitro studies revealed that galectins prevent the attachment of virus into fish epithelial cells (Nita-Lazar et al. 2016). Lectins prevent viruses from binding to the surface portions of cells and also inhibiting the proliferation of the viruses. Virus uptake of some cells can be accelerated by some lectins known as Ss Lec 1, are C-type lectins purified from a Black Rockfish, *Sebastes schlegelii*, which causes an increase in an infectious spleen and kidney necrosis virus (ISKNV), and the copy numbers found in these fishes are also in the presence of copy numbers in black rockfish, also in the presence of SsLec1 bacteria were actively undergone in phagocytosis and the kidney macrophages were able to kill (Liu et al. 2016). AJL-1 galectin molecules isolated from a Japanese eel are able to inhibit the process of biofilm formation by *Aggregatibacter actinomycetemcomitans*, Y4 ATCC-29523 strain and ATCC-29524 strain, which can cause periodontal disease in humans (Takayama et al. 2009). Similar study on Galectin AJL 1 reported its capability to inhibit the biofilm formation of the teleost lectins (Takayama et al. 2009) and its uses in the treatment of Periodontitis was also reported. L-Rhamnose-binding lectins, STL, was isolated and purified from the Steelhead Trout Egg and constituted the result as the lipopolysaccharides and the lipoteichoic acid are able to agglutinate *E. coli* strain K12 and inhibit the binding ability of *Bacillus subtilis* by a L-Rhamnose (Tateno et al. 2002). A study on ES-Lec molecules, which were isolated and purified from Pearl spot (Etroplus suratensis) serum has able to inhibit biofilm formation. In addition, these lectins show widespread antimicrobial activities, and on the bacterial species mainly on Gram-negative organisms like *Aeromonas hydrophila* and *Vibrio parahaemolyticus*, they also disrupt the architecture of biofilm (Rubeena et al. 2019). The functional activities of purified lectin molecules Md-Lec, from haemolymph of *Metapenaeus dobsoni,* exhibit the haemagglutination activity with human erythrocytes and showed antibacterial activity against *Aeromonas hydrophila* and *Vibrio parahaemolyticus* (Rubeena et al. 2019).

A study on lectins which were coated with nanoparticles and subjected to fishes after the experimental infection revealed that they showed more resistance against infections, also the genes expressed after infection within a short period. In this work, Md-Lec was conjugated with CuS Nanoparticles, and it showed more inhibition effect on 100 μg/mL which was demonstrated on agar diffusion method. At the concentration of 100 μg/mL Md-Lec, the biofilm formation by *A hydrophila* was arrested completely. This study concluded that the methods used can be able to produce economically important and disease-free fishes after further molecular and immunological studies with the immunostimulant nanoparticles (Preetham et al. 2021). The best delivery systems in microbes are capable of preventing or decreasing the degradation reaction including biological or chemical processes, site specificity and preventing the binding with serum proteins (Nordström and Malmsten 2017). The drugs from macromolecules like these proteins are delivered with the nanoparticles constituted by inorganic materials such as silica, clays, metals, the

carbon-based materials and also some of the polymers like fibres, micro gels, multi layers and conjugates. Some of the stabilizing agents can be attached to the nanoparticles which are able to prevent a chemical or biological degradation, and the targeted ligand can help antimicrobial agents to reach and act on specific sites.

13.4 Role of Lectin in Hemagglutination

In Agglutination, the molecules are able to adhere or form clumps and involve in inducting the mechanism of adhesion is defined as agglutinins, which are the proteins that are able to agglutinate. The antibodies are the portion of the adaptive immune system, also are the classical agglutinins (Boes 2000). The haemolymph of the Speckled Shrimp *Metapenaeus monoceros* contains a lectin Mm-Lec, which has the ability for hemagglutination process using human RBC cells and has an agglutination ability against some of bacterial species like *Saccharomyces cerevisiae* yeast agglutination activity against *Saccharomyces cerevisiae* which these performance of lectins in erythrocytes were analysed with a light microscopy (Preetham et al. 2019).

The lectin molecule from the Green Tiger shrimp, *Penaeus semiculcatus*, *Ps*-Lec was able to take part in the haemagglutination procedure with respective erythrocyte cell in vertebrates (Sivakamavalli and Vaseeharan 2014). Numerous mucosal lectins are able to agglutinate with bacterial species and hemagglutination with non-self-erythrocytes. In mucous and mucosal surfaces of gill, the mechanism of agglutination plays a crucial role in the uptake of the pathogenic organisms. The agglutination process at the time of swimming can let this pathogen fall off, and in case of pathogen present in the gastrointestinal tract will be expelled out with the faeces. The viscous mucous which contains immunologically applicable novel molecules besides these lectins are competent to agglutinate with microbes and also non-self-erythrocytes (Brinchmann 2016). Various proteins found in mucosal layers are involved in the work with the mechanism of agglutination, and lectins alone can execute hemagglutination and agglutination (Brinchmann et al. 2018).

Agglutination capability of lectins in mucosal cells among the others for the Galectin 1, from Atlantic cod (Rajan et al. 2011), AJL1 Galectin from a Japanese eel (Tasumi et al. 2004) were studied and C-type lectins AJL2 (Tasumi et al. 2002) and also C-type lectin from the same fish (Liu et al. 2016). Similar studies reported in various lectins on Ss LtL, Lily-type lectin from a Black Rockfish, *Sebastes schlegelii* (Kugapreethan et al. 2018), Galectin Congerins isolated from a Conger eel (*Conger myriaster*) (Tasumi et al. 2002), Cs LtL 1 from Stripped murrel, *Channa striatus* (Arasu et al. 2013) rOnMBL lectins from a Nile Tilapia, *Oreochromis niloticus* (Mu et al. 2017) and pufflectin from Pufferfish (*Takifugu rubripes*) (Tsutsui et al. 2003). Congerins from Conger eel are able to induce only the agglutination process of *Vibrio anguillarum*, which was purified from a mucus cells of skin; they are not able to inhibit or lysis of the bacterial pathogens (Kamiya et al. 1988). Hemagglutination is also common among mannose-binding netterin like proteins isolated from an Atlantic Cod fish, *Gadus morhua* in a mode with calcium ions dependant (Rajan

et al. 2017), for the mannose-binding SsLTL, lily-type lectin from a Black rockfish, *Sebastes schlegelii* (Kugapreethan et al. 2018) and a mannose-binding lectin molecule from a flat head, *Platycephalus indicus* which has a similar kallikrein (Tsutsui et al. 2011a, b).

Some of the researchers reported a study on the natural hemagglutinins found in common freshwater habitat Indian catfish; *Clarias batrachus* and a *Heteropneustes fossilis* with their anti-erythrocyte were studied and possessed haemagglutinins against erythrocytes of humans. Serum hemagglutinins present in both species are able to agglutinate the erythrocytes with ABH determinants; here, the Lewis system and some unknown epitopes were found in surfaces. These hemagglutinins are capable of reacting with a Oh Bombay phenotype red blood cells and also agglutinate with amphibian and avian red blood cells and were treated with enzymes and lacking ABH group determinants. The ability of a hemagglutinin molecule isolated from Catfish has fit to bind with rabbit erythrocytes was inhibited by a melibiose, the study revealed that the serum of Indian Catfish which contains different types of hemagglutinins (Dash et al. 1993). The lectin molecules KPL from a Skipjack Tuna fish, *Katsuwonus pelamis*, show a specificity in agglutination against the type A RBC cells of humans (Jung et al. 2003). In another study on lectins, CTL and HjCL were isolated and purified from *Heterodontus japonicus,* which is a Japanese Bullhead Shark; these lectins have an ability for binding with various sugar compounds also and induced the process of blood coagulation process (Tsutsui et al. 2015).

A Lectin molecule with immunological properties MmLec isolated from *Metapenaeus monoceros*, is a Speckled Shrimp, haemolymph subjected to functional analysis and concluded that, MmLec had haemagglutination activity against erythrocyte cells of human beings and also a yeast agglutination activity against a yeast, *Saccharomyces cerevisiae* (Preetham et al. 2019). Shrimp lectins from *Litopenaeus setiferus* (Zenteno et al. 2000) and *Fenneropenaeus chinensis* (Sun et al. 2008) exhibited related features of agglutination on the human RBC. The lectin molecules from a shrimp plasma from *Fenneropenaeus chinensis* was isolated, also purified and showed the presence of strong affinity for the erythrocytes of human including A, B, O groups, mouse and chicken red blood cells too (Sun et al. 2008). A molecule of lectin from *Oncorhynchus ketaan*, egg chum Salmon, has various patterns of hemagglutination in the erythrocyte cells of rabbit, explained with the usage of lipopolysaccharides from *E. coli, Aeromonas salmonicida* and *Bacillus subtilis* (Shiina et al. 2002). The lectins or haemagglutinins and lectin-like molecules which were also found in the mucus cells on the surface of the skin of fishes are involved in various immunities like innate and acquired immunity. Agglutinins in fishes are reported; they are healthy to prevent the polyspermy also involved in the process of wound healing. When parasitic infection occurs in a fish, the level of lectins was increased. One of the research works on the natural serum lectins or hemagglutinins from belonging to the major carp family, *Cirrhina mrigala,* found that they demonstrate that these lectins are able to agglutinate with the rabbit red blood cells and also to lyse the RBC (Saha et al. 1993). Haemagglutination study of C-type lectins from Pearl Spot fish, *Etroplus suratensis*, reported that purified

Es-Lec had the agglutination activity with human RBC cells, highest agglutination was shown at 100 μg/mL, the lowest was at 25 μg/mL, and the maximal level of haemagglutination activity was found at the temperature of 4 °C and at 25 °C also at 10 mM Ca ion concentration (Rubeena et al. 2019).

13.5 Role of Lectins in Endocytosis and Phagocytosis

The phenomenon of phagocytosis is an actin-dependent process, involving the ingestion of larger molecules by phagocytic cells. Elie Metchinikoff was the first to describe phagocytosis and get awarded a Nobel Prize in Physiology and Medicine in 1908. Phagocytic cells participate in various biological processes, like control and recognition of invading microbes, and the recognition procedure was mediated by a germline-encoded recognition receptor cells (PRPs). These membrane-bounded PRPs are soluble and are able to recognize sources of microbes called Pathogen-Associated Molecular Patterns (PAMPs), like bacterial lipopolysaccharides or the β-glucan fungal pathogens (Janeway and Medzhitov 2002). The germline-encoded PRPs can bind to the microbial structures participating in the activation of the defensive mechanism of the host, which includes the intake of microbes through phagocytosis, when recognizing and responding to pathogens. A lectin molecule exhibits an affinity towards the carbohydrate molecules, capable of agglutination with cells or of precipitation of glycoconjugates. These molecules show an antimicrobial potential in the skin mucus and deeply interact with the surface of pathogenic organisms which result in opsonization, enhancing the phagocytosis (Ottinger et al. 1999), or activation of the complement pathway (Matsushita et al. 2004).

C-Type lectin molecules are calcium-dependent molecules with carbohydrate-binding proteins have a conserved recognition domain of carbohydrate, the same other proteins containing the same structural domain which cannot bind with carbohydrates or calcium molecules. These C-type lectins are a superfamily of a large protein class with the presence of one or more than one C-type lectin domain and are classified into 17 large groups on the basis of phylogenetic and domain organizational studies (Zelensky and Gready 2005). C-type lectin is diverse in its functions and enzymatic activities, involved in different processes like tissue integration and remodelling, cell adhesion, pathogen recognition, platelet activation, complement activation, and phagocytosis and endocytosis.

Conger myriaster, a Conger eel, has a C-type lectin, which tightly binds to microspheres and increases the phagocytic action in their macrophages (Tsutsui et al. 2007). The Opsonization activity of Salmon serum lectins were reported, which has acts as an opsonin molecules for *Aeromonas salmonicida* and involved in the process of phagocytosis of a heat-killed *A. salmonicida* by macrophage cells (Ottinger et al. 1999). Serum lectins, isolated from a Sea Bass, *Dicentrarchus labrax*, its intestinal cells and hepatocytic localizations and the expression were reported; the exposure of *E. coli* cells enhanced the phenomenon of phagocytosis by a peritoneal macrophages in *Dicentrarchus labrax* (Salerno et al. 2009). The lectins from the

serum of a Gilt head Bream, *Sparus aurata* species, bind with a formalin-killed *E. coli* strain and also take part in the enhancement of phagocytic action by macrophages in the peritoneum (Cammarata et al. 2012). These lectins also serve and act as opsonins, also playing a valuable role in innate immunity. Mm-Lec isolated from a haemolymph of speckled shrimp *Metapenaeus monoceros* is able to involve in the identification of foreign antigens efficiently with the help of pathogen-associated molecular patterns, PAMPs, also PRPs. These activities can lead to the elimination of a pathogen from the host by the action of phagocytosis (Preetham et al. 2019).

The F-type lectins isolated from a Sea Bass, *Dicentrarchus labrax*, were localized and also expressed in the intestinal and liver portions, which enhanced the fucose inbitable and an taking up of *E. coli* cells by the process of phagocytosis by macrophages in peritoneum (Salerno et al. 2009). Galectin-1 from Atlantic cod seemed to have phagocytosed latex beads (Rajan et al. 2013), which points out that phagocytosis is stimulating the lectin molecules to stimulate phagocytosis at the time of bacterial infections. Galectins from an Atlantic cod from *Francisella noatunensis*-infected fish found that they are acting as opsonization agents or capability to kill the pathogenic groups (Rajan et al. 2017). Lectins share immunological activities and play a main role in disease protecting systems, also a role as opsonins, endocytic uptake of pathogens, promoting phagocytosis (Brinchmann et al. 2018). A similar study on mRNA rhamnose-binding lectins, early after *Flavobacterium columnare* infection, these lectins are found to be able to involve the reaction, these lectins are present in the gills of a catfish, *Ictalurus* sp. (Sun et al. 2012). The catfishes are not sensitive to *F. columnare*, while in some fishes *Flavobacterium columnare* causing infections are inhibited by the D-galactose and L-rhamnose binding lectin ligands (Beck et al. 2012). Another study in rhamnose binding lectins was reported in Chum Salmon, *Oncorhynchus keta* binds with globotriaosylceramide which encourages phagocytosis (Watanabe et al. 2009). Globotriaosylceramide, well known as receptor for some serogroups of *E. coli* and shiga toxin B subunit of *Shigella dysenteriae* and also stimulating the clathrin-dependent uptake of toxin endocytic action (Sandvig et al. 1989). The C-type lectin from Blue Gourami, *Trichogaster trichopterus,* BGL, had a key role in the enhancement of innate immunological activities against *Aeromonas hydrophila*, and these lectins were combined with laminarin (Beta1, 3 D-glucan) and show the intake of bacterial strains by the macrophages (Fock et al. 2001). Study on natural serum lectins or haemagglutinin revealed that serum of *Clarias batrachus* and *Heteropneustes fossilis* had the presence of opsonins, which is able to stimulate the macrophages in rat peritoneum to engulf a bacterium, *Staphylococcus aureus*. This study concluded with the results of defence mechanism of fishes and has a powerful humoral immune responses, which help them to protect themselves from pathogens (Saha et al. 1993).

The Mannose binding lectins are able to form the structural unit of polypeptide chains, binds with carbohydrates which founds in the microbial surfaces by C Terminal lectins, which can involve in phagocytic process or killing purposes (Whytev 2007). Some of the studies reported that mannose-binding lectin molecules

are able to bind and can agglutinate with Gram-positive and Gram-negative bacterial species including mycobacterium also with viruses and fungi (Tsutsumi et al. 2005). Several studies were reported on mannose-binding lectins. The fish lectins from Salmon were structurally and immunologically characterized and found to be susceptible to various pathogens, and these lectins are responsible for the enhancement of the activity of macrophages (Ewart et al. 2001). Researchers studied on various types of mannose binding lectins from different fishes and confirmed the ability of mannose-binding lectins in pathogen-binding activity and also in complement pathway, and studies are carried out on fishes like Rohu (*Labeo rohita*), Sea Lamprey (*Petromyzon marinus*), Atlantic Simon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss)*, Fugu (*Takifugu rubripes*), Common Carp (*Cypris carpio*) and Turbot (*Scophthalmus maximus*) (Zhang et al. 2009). Channel catfish (*Ictalurus puntatus*) is a common and important Aquaculture species, the mannose binding lectins and studied the binding activity of lectins with its resistance against *Edwardsiella ictaluri* (Ourth et al. 2007). They also studied gene expression and cloning and characterize the mannose binding lectin gene. Some of the research studies demonstrated the multiple copies of mannose-binding lectin found in Zebrafish and assessed the copy number variations, and the polymorphism can affect resistance against pathogenic infections (Jackson et al. 2007).

Mannose-binding lectin (MBL) in several fishes has been reported with the presence of Pattern Recognition Receptor and the involvement of activity factor in the complement system (Nauta et al. 2003). In fishes, several studies confirmed the binding ability of mannose-binding lectins to carbohydrates on the surfaces of pathogens, and this binding capability has an ability to activate the complement system or directly involve as opsonins. The MBL expression analysis disclosed that these MB lectins were mostly expressed in the liver cells of a Catfish. In the case of Rainbow Trout (*Oncorhynchus mykiss*), Trout MBL1 was expressed in liver cells and MBL2 in spleen cells (Nikolakopoulou and Zarkadis 2006). Related study on these types of MBL in Common Carp (*C. carpio*) showed the expressions in spleen and liver (Gonzalez et al. 2007). In Fugu (*T. rubripes*), the pufflectin gene shows gene expressions in oesophagus, skin, intestine, cavity walls, etc. (Tsutsui et al. 2005). The study concludes that channel catfish share structurally similar elements with the mammalian MBLs, which indicates the evolutionary relationship on motif and domains mutations.

C-type lectins are Pattern Recognition Receptors (PRPs) which were isolated and characterized from a Tiger shrimp (*Pinnaeus mondon*) and named as PmCL1, and its mRNA transcript was highly expressed in hepatopancreas; this expression was upregulated by *Vibrio harvei* and *V. anguillarum.* The PmCL1 specificity to pathogen-associated molecular patterns including peptidoglycan, galactosamine, α-lactose trehalose, D-mannose, and D-glucose were explained by carbohydrate-binding assay. A recombinant PmCL1 which agglutinated *Staphylococcus aureus* and Gram-negative *Vibrio harvei* and *V. anguillarum, V. iginolyticus, V. vulnificus* and *Aeromonas hydrophila* enhanced the complete removal or inhibition of invading bacterial specie in the presence of calcium ions. Results of this study concluded that PmCL1 has an important role in Pattern Recognition Receptor in immune functions towards infectious diseases (Qiu et al. 2011).

13.6 Conclusion

Lectins are a promising group of biomolecules which encompass antitumor, antifungal, antiviral, antibacterial, anti-nematode, anti-insect and immunoregulatory activities. Fishes offer a diverse emporium of lectins which have an array of potentials like agglutination, pathogen recognition, complement activation, opsonization, phagocytosis and other functions like RNA splicing, molecular trafficking, protein folding and cell proliferation control. Although diverse applications have been accomplished for fish lectins, the accessible information on the underlying mechanism is scanty and provides a scope for the tremendous research in this field.

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Part V
Therapeutic Effects of Aquatic Lectins

Chapter 14
Functional Aspects of Fish Mucosal Lectins and Crustaceans with Its Applications

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Abstract Fish skin mucus is an important part of the innate immune system that acts as a first line of defence against infections. Because of the presence of gel-forming macromolecules and hyaluronic acid, mucus has a slippery texture. Lectins, which are a key component of mucus, are carbohydrate-binding proteins that are neither antibodies nor enzymes, thus far taking part in vital roles in both innate and adaptive immunity. Fish mucus lectins are categorized into four types and mucus lectins have recently been studied for their structural variety and involvement in innate immunity. The identification, types and applications of fish mucus lectins are reviewed in this chapter.

Keywords Mucus · Lectin · Fish · Agglutination · Antibacterial activity · Applications · Phagocytosis

Abbreviations

AJL	*Anguilla japonica* lectin
AMP	Antimicrobial peptides
conCL	Congerin lectin
CRD	Carbohydrate recognition domain
CsLTL	*Chana striata* lily-type lectin
FAO	Food and Agriculture Organization

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FBL	Fucose-binding lectin
FHL	Flat head lectin
FTLD	Fucose recognition domain
GBL	Galactose-binding lectin
kDa	Kilodalton
LBL	Lactose-binding lectin
MAAs	Mycosporine-like amino acids
Nlp	Natterin-like protein
NOAA	National Oceanic and Atmospheric Administration
PFL	Puffer fish lectin
PRRs	Pattern recognition receptors
RbFTL	Rock bream F-type lectin
RBL	Rhamnose-binding lectin

14.1 Introduction

The foremost agency for aquaculture, the National Oceanic and Atmospheric Administration (NOAA) of United States of America has committed federal guidance and financial aid to the states in order to generate aquaculture regulation, strategy and physical systems. Aquaculture is described by the NOAA as 'the production and rearing of aquatic organisms in regulated or selected aquatic habitats for any economic, cultural, or public purpose'. Aquaculture and fisheries are important sectors for food supply and economic development, with a wide range of resources and possibilities. As per the Food and Agriculture Organization (FAO), over the next 30 years, the supply from capture fisheries will remain constant. Aquaculture accounts for a growing percentage of global aquatic production, and its relevance is anticipated to grow drastically as a result of global overfishing and rising seafood consumption (FAO 2010). Indeed, aquaculture sector has grown from 9% of fisheries resources in 1980 to 43% now, with the FAO estimating that productivity will double in the next 25 years. Aquaculture is promoted by the FAO not only as a valuable source of revenue and employment but also as a significant contributor to food security and social welfare in many countries (FAO 2016). Alternative preventive strategies are needed in the modern aquaculture sector to ensure good animal welfare and a healthy environment, resulting in improved productivity and revenues. These goals will be easier to fulfil if we have a better understanding of the immune system of farmed fish (Esteban 2012).

Fish, as a phylum, have contributed to a better understanding of immune system evolution since their immune systems are physiologically parallel to those of higher vertebrates (Uribe et al. 2011). The fish immune system, like that of mammals, is divided into innate and acquired immune responses. Innate immunity has a broader and robust response, whereas adaptive immunity has a very particular response to infectious organisms (Magnadóttir et al. 2001; Magnadóttir 2006, 2010), and

adaptive response is frequently delayed, yet it is critical for long-term immunity (Secombes and Wang 2012). Fish rely on their innate immune system for survival because they are free-living organisms from the beginning of their lives. Fish come into constant touch with a diverse range of non-pathogenic and pathogenic microbes in the aquatic environment, thus they have evolved mechanisms to ensure their survival. Compared to air, water has a higher quantity of microorganisms such as bacteria, fungus and protozoa. Fish skin is constantly exposed to a harmful environment, but it lacks a stratum corneum and is made up of living cells (Tsutsui et al. 2011a). As a result, the epidermis is a high-risk area for microbial attack. Fish are thought to have a unique defence system in their skin surface that differs from that of land vertebrates. Skin mucus is an essential element of fish innate immune system, serving as a first physical and chemical barrier towards infections, and hence plays a critical role in fish health (Subramanian et al. 2007). Mucosal immunity in fish is a little-studied research subject, but there is a lot of interest in it right now. The mucosal immune response act as a vital part in the route of illness since the majority of infectious agents target or initiate infection on mucous surfaces (McNeilly et al. 2008), and various studies have begun to explore their cellular and molecular makeup in various species (Palaksha et al. 2008; Rombout et al. 2008; Rajan et al. 2011; Zamzow 2007; Purcell and Anderson 1995; Sugiyama et al. 2005). This chapter will discussed as a highly important part of the bioactive protein lectin from the fish skin mucus and serves as a crucial aspect of their immune system, and a brief outline of the subject. This is a vast issue, so it will take a while, and is not intended to be a comprehensive study, but rather an attempt to describe the identification of lectin from fish mucus and its applications in this field. This is a field of study that dates back to the 1960s and is still relevant today for a variety of reasons that will be discussed.

14.2 Mucus

The presence of external mucus is one of the most distinguishing characteristics of fish skin. Owing to the occurrence of gel-forming macromolecules and elevated water content, mucus has a slippery texture (Shephard 1994). The external mucus gel coats the live epithelial cells with an adhesive mucus layer, and it serves as a thin barrier between the fish and its watery surroundings. The fish skin is constantly vulnerable to pathogen attack because water is a great habitat for bacteria and parasite microorganisms. It is widely assumed that a pathogen defence system exists on the surface of the fish body and that skin mucus serves as both mechanical and biochemical barrier. Mucus is thought to be the epidermis' initial line of defence against infection (Raj et al. 2011). Most germs and pathogens cannot pass through skin mucus. Foreign particles, bacteria and viruses are captured by mucus and evacuated from skin mucosa by the surrounding water. Innate immune components released by goblet cells in fish epidermal mucus constitute the major defence against destructive pathogens and perform the hurdle among the fish and its local habitat.

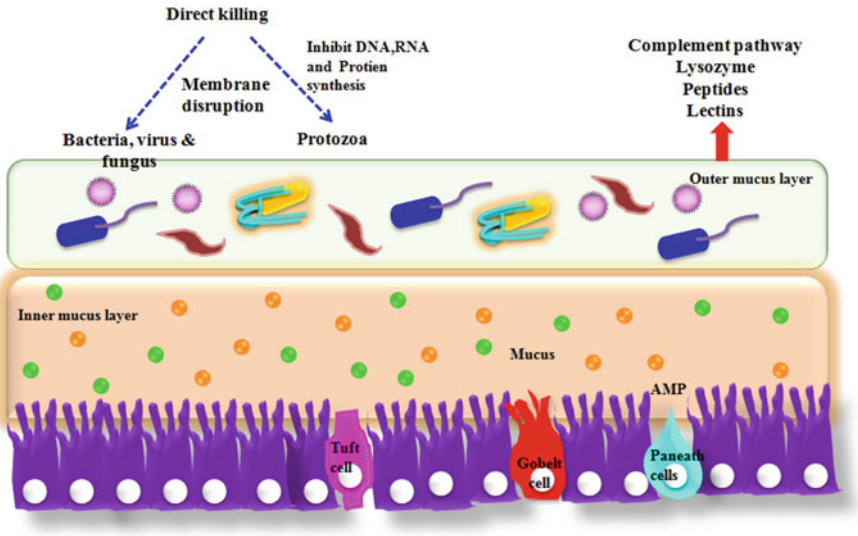

Fig. 14.1 Schematic representation of fish mucus

Entrapment and sloughing of bacteria are two main functions of mucus. The schematic representation of fish mucus is shown in the Fig. 14.1. It is a substance that aids reproduction, swimming, excretion, communication, disease resistance, feeding, ionic and osmotic management, and nesting. Proteins, carbohydrates, lipids and metabolites are all found in the skin mucosa of fish (Zaccone et al. 2001). Depending on the species, skin mucus has a wide range of viscosity, thickness, and glycoprotein (mucin) concentration, which is one of the most important components of mucus (Dash et al. 2018). Many essential proteins and enzymes have been identified in fish mucus including protease, lysozymes, antimicrobial peptides (AMP), glycosaminoglycans, immunoglobulins, complements, carbonic anhydrase, transferrins, lectins, calmodulin and antibacterial peptides (Shoemaker et al. 2005; Swain et al. 2007). The identification of various bioactive compounds and their potential application in human health and aquaculture has boosted fish mucus research in the last 10 years. The majority of fish mucus research has thus far focused on immune-related chemicals and AMPs, but few studies have looked into additional mucus molecules and their ecological significance in the environment. As a result, the current chapter examines the many forms of lectin found in fish mucus, their properties and activities, biological significance, and prospective aquaculture applications.

14.3 Lectin

Lectins are carbohydrates, proteoglycans, glycolipids, and glycoprotien-binding proteins. Lectins are phylogenetically extremely older and have the capacity to identify and attach to complex carbohydrates found in glycoconjugates. Lectins were first found in plants over a century ago. It is currently recognized to exist in a wide range of organisms, including bacteria, viruses, plants and animals (Gupta 2012). The specificity of their carbohydrate-binding domain and their reliance on divalent cations for their activities (Brinchmann et al. 2018). Lectins are essential immunological mediators in lower vertebrates and invertebrates, have been discovered in fish skin mucus and may play a role in innate and acquired immunity. In mucus, lectins can be present in both intra- and extracellularly. They are released into mucus through a variety of mechanisms, including the conventional endoplasmic reticulum/Golgi apparatus secretory pathway, in which the protein is co-translationally synthesized on the endoplasmic reticulum and then transported to the cell surface (Chua et al. 2012). These proteins are components of the innate immune system that have a partiality for carbohydrate moieties, in addition to cell agglutination and glycoconjugate precipitation. They have the ability to bind carbohydrates, which are necessary for cell wall adhesion. They may have antibacterial effect in the skin mucus because of its characteristic. Today, lectins are a diverse set of carbohydrate-binding proteins generated from the non-immune system that agglutinate cells and precipitate glycoconjugates devoid of distressing their covalent associations (Goldstein et al. 1980). According to this description, lectin molecules have two or more carbohydrate-binding sites that allow the cell to crosslink with sugar-containing macromolecules. Despite the fact that lectins are not produced by the immune system, their agglutination power is comparable to that of antibodies. Apart from being able to distinguish between distinct monosaccharides, lectins may also detect minute differences in complex carbohydrate structures and selectively attach to oligosaccharides. Lectins attach to pathogen surface features and can either opsonize and improve phagocytic activity or activate the complement system (Matsushita et al. 2004).

14.4 Identification

Lectins are a class of non-enzymatic carbohydrate-binding proteins that identify mono or oligosaccharides moieties in their ligands. Although lectins were first purified and identified from plants, they are found in a wide range of organisms from microbes to vertebrates, including teleosts and other aquatic creatures, and play a role in a variety of biological processes. Lectins have also been found in the skin mucus of fish species, and they play a vital defensive role on the body's surface. Several types of lectins, including various forms of lectins, have been discovered in the mucus of fish skin are presented in Table 14.1. Lectins are classified into four

Table 14.1 Identification and types of lectins from fish mucus

Fish species	Lectin family	Specific binding site	Molecular weight (kDa)	References
Pink cisk eel (*Genypterus blacodes*)	Lectin	–	32	Oda et al. (1984)
Arabian Gulf catfish (*Arius thalassinus*)	GBL	Galactose	20	Al Hassen et al. (1986)
Conger eel (*Conger myriaster*)	Congerin	Galactose	13	Kamiya et al. (1988)
Conger eel (*Conger myriaster*)	GBL	Galactose	12	Shiomi et al. (1989)
Dragonet (*Repomucenus richardsonii*)	Acidic glycoprotein	–	48	Shiomi et al. (1990)
Kingklip (*Genypterus capensis*)	Mitogenic lectin	–	28–34	Toda et al. (1996)
Loach (*Misgurnus anguillicaudatus*)	MAL-1	–	40 and 41	Goto-Nance et al. (1995)
Conger eel (*Conger myriaster*)	Congerin II	–	–	Muramoto et al. (1999)
Conger eel (*Conger myriaster*)	Lectin	β-Galactoside	30	Muramoto and Kamiya (1992)
Japanase Conger eel (*Conger myriaster*)	Galectin	β-Galactoside	–	Nakamura et al. (2001)
Japanese eel (*Anguilla japonica*)	AJL-1 and AJL-II	Lactose	16 and 31	Tasumi et al. (2002)
Ponyfish (*Leiognathus nuchalis*)	RBL	Rhamnose	–	Suzuki et al. (2003)
Pufferfish (*Fugu rubripes*)	Pufflectin	Mannose	13	Tsutsui et al. (2003)
Japanese eel (*Anguilla japonica*)	AJL-1	Ca^{+2}-dependent β-galactoside	30	Tasumi et al. (2004)
Ponyfish (*Leiognathus nuchalis*)	(PFL-1 and -2)	Lactose	24 and 30	Okamoto et al. (2005)
Conger eel (*Conger myriaster*)	conCL	Mannose	16	Tsutsui et al. (2007)
Cartilaginous fish (*Raja kenojei*)	LBL	Lactose	25	Tsutsui et al. (2009)
Atlantic cod (*Gadus morhua*)	Galectin-1	Mannose	–	Rajan et al. (2011)

(continued)

Table 14.1 (continued)

Fish species	Lectin family	Specific binding site	Molecular weight (kDa)	References
Catfish (*Silurus asotus*)	Intelectin	Ca^{+2}-dependent mannose	35	Tsutsui et al. (2011a)
Indo-specific flat-head (*Platycephalus indicus*)	FHL	Ca^{+2}-dependent mannose	40	Tsutsui et al. (2011b)
Channel catfish (*Ictalurus punctatus*)	RBL	Rhamnose	–	Thongda et al. (2014)
African catfish (*Clarias garipinus*)	GBL	Galactose	63	Odekanyin and Ku KU (2014)
European sea bass (*Dicentrarchus labrax*)	FBL	Fucose	–	Cordero et al. (2015)
Giant mottled eel (*Anguilla marmorata*)	AJL-1	–	–	Tsutsui et al. (2016)
Atlantic cod (*Gadus morhua*)	Nlp	Natterin-like protein	35	Rajan et al. (2017)
Loach (*Misgurnus anguillicaudatus*)	LML	–	245	Sun et al. (2019)

classes based on the structure of the carbohydrate recognition domain: C-type, galectins, P-type, and I-type.

14.5 Types of Lectin

Several types of lectins, including different forms, have been identified in fish skin mucus. On the basis of the structure of the carbohydrate recognition domain, animal lectins are divided into four main groups; they are as follows.

14.5.1 Mannose-Binding Lectins (MBLs)

MBL is an important component of innate immunity that is capable of activating the complement system lectin pathway and plays a key role in host's first line of defence. They can detect antigens from bacteria, parasitic protozoa and fungus, among other organisms. Furthermore, it has the ability to resist self-recognition and accomplish the result of 'alien' identification in order to safeguard 'themselves' (Eddie Ip et al.

2009). MBLs are C-type lectins that bind to mannose moieties in microbial pathogens and opsonize them, triggering phagocytosis and the complement pathway. Other functions include agglutinating bacteria, scavenging cellular detritus, cell–cell communication and acting as inflammatory mediators and immune cell mitigens.

14.5.2 Rhamnose-Binding Lectins (RBLs)

RBLs are Ca^{2+}- independent lectins that recognize L-rhamnose and D-galactose and are made up of one or more homologous carbohydrate recognition domains (CRDs) with a distinct fold. Teleost, tunicates and other aquatic invertebrate species like bivalves and sea urchins have been largely isolated from RBLs. RBL family members have been found in more than 25 different fish species, with expression mostly in the ovaries, eggs and skin mucus (Ogawa et al. 2011). In the innate immune response, RBLs have been shown to act as an antibacterial and non-self-recognition molecules. The CRD structure is an important element in lectin categorization. Each RBL CRD is made up of roughly 100 amino acids that encode eight conserved cysteine residues, resulting in four disulphide bridges in its distinctive topology (Ballarin et al. 2013). The vast fish and invertebrate RBL repertoire has been assigned a wide range of functional tasks. They include glucose metabolism management, fertilization control, cytotoxicity and immunity.

14.5.3 Fucose-Binding Lectin (FBLs)

F-type of lectins are fucose-binding proteins that are found in a wide range of taxonomic groups, from viruses to vertebrates, and are mainly newly revealed lectin family. They have a fucose recognition domain (FTLD) by a new fold that consists of a barrel with jellyroll topology and exclusive fucose and calcium-binding sequence motifs (Bianchet et al. 2002). Although FTLs can have a single FTLD, which is at times attached through one or more structurally and functionally diverse domains in a single polypeptide, members of this lectin family can also have a variable number of tandemly arrayed FTLDs. FTLs play a role in immunological detection, while others play a role in microbial pathogenesis, fertilization and a variety of other tasks (Vasta et al. 2017). Moreover, fucose-binding lectin (FBL) identifies carbohydrates on the surface of potential pathogens, causing phagocyte activation through agglutination, immobilization and opsonization of microbial pathogens.

14.5.4 Galactoside-Binding Lectins (GBL)

Galectins are a large family of lectins that are found in a wide range of multicellular animals and fungi and engaged in a wide range of biological process, and growing evidence suggests that they play a role in immunological processes. Based on their fundamental architecture, they are currently divided into three categories: proto-type, tandem repeat-type and chimera-type. Galectins of the proto-type are made up of two identical 14–16 kDa subunits that share a single carbohydrate recognition domain (CRD). More than 10 primary structures of proto-type galectins from mammals, birds, amphibians, fish, nematodes, sponges and fungus have been found yet. Furthermore, new discoveries have revealed indications of galectin–infectious organism interactions. Galectin-1 binds to Nipah virus and HIV envelope glycoproteins, whereas galectin-3 attaches to mycobacterium TB and has a role in late mycobacterial infections (Levroney et al. 2005). *Trypanosoma cruzi* adheres to human vascular smooth muscle cells via Galactin-3 (Kleshchenko et al. 2004). Galectins appear to operate as pattern recognition receptors (PRRs) for a variety of pathogenic pathogens, according to these findings. Congerin is a proto-type galectin identified in the skin mucus of the Japanese conger eel *Conger myriaster*. It is made up of two identical subunits. It also functions as an opsonin, as evidenced by a substantial increase in the rate of latex microsphere ingestion by peritoneal macrophages when the beads were pre-coated with recombinant congerin I. When the congerin-coated beads were pre-incubated with lactose, the effect was considerably diminished, indicating that congerin-peritoneal macrophage binding is dependent on the interaction between lectin and galactosides.

14.6 Applications

Lectins have a variety of uses in biological research, including as agents and tools. It could be employed as anti-insect, antifungal, antiviral, anticancer medicines according to several reports. Despite the fact that lectins have been used in a range of applications in many research and review publications, the applications of fish mucus lectin has not yet been systematically addressed, and this problem is discussed in this chapter. Lectins are components of the innate immune system that have an affinity for carbohydrate moieties and can cause cell agglutination and glycoconjugate precipitation. They may have antibacterial effect in the skin mucus because of this characteristic. Lectins bind to pathogenic surface features, causing opsonization, increased phagocytic activity or complement pathway activation. Agglutinins in fish have also been to inhibit polyspermy and aid wound healing. During parasite infection, lectin levels in fish mucus have been shown to increase. Furthermore, the schematic diagram of fish mucus lectin applications were shown in the Fig. 14.2.

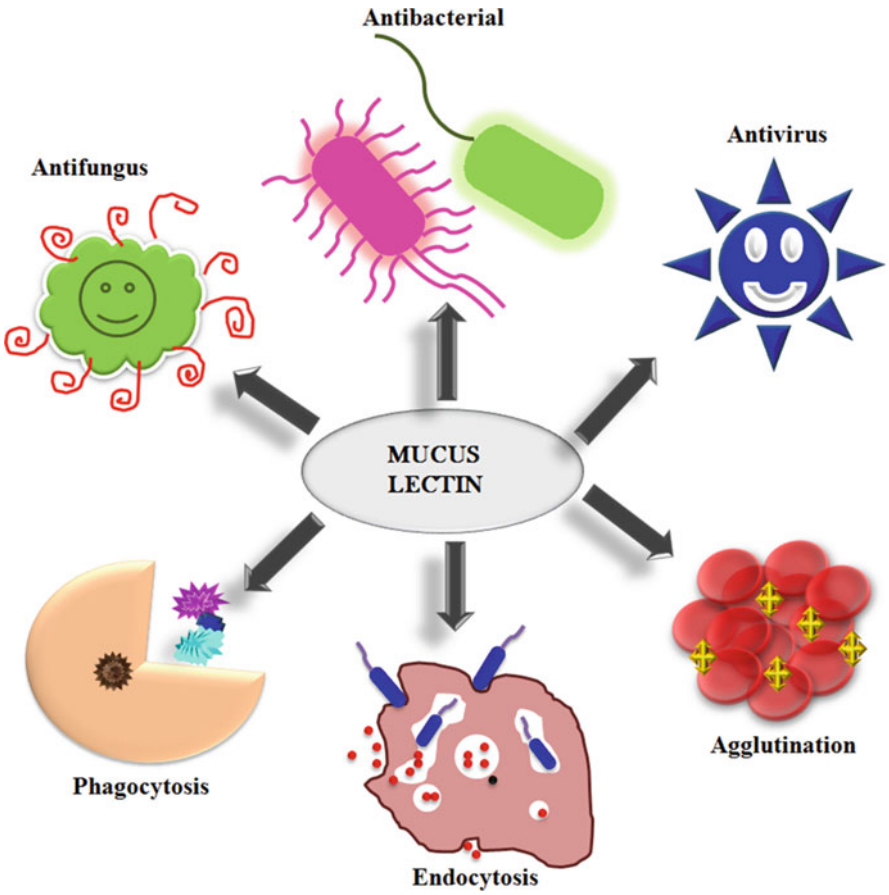

Fig. 14.2 Different types of applications in mucus lectins from fish

14.6.1 Lectins Act as a Agglutination Reaction

Lectins are one of the most significant agglutinins in the innate immune system. Many mucus lectins agglutinate bacteria and non-self-erythrocytes. Haemagglutination is the process of agglutinating non-self-erythrocytes. When non-self-viruses, −bacteria, −yeast and -parasites bind to lectins, they can agglutinate, be targeted for destruction (via complement activation or endocytic/phagocytic uptake), or be destroyed directly by the lectins. Mechanisms like agglutination are vital in avoiding pathogen uptake on mucus surfaces. Agglutination in the skin and gill mucus layers can cause pathogens to slip off during swimming, and pathogens in the gastrointestinal system can be evacuated with the faeces (Brinchmann 2006). Apart from lectins, mucus contains a variety of immune relevant compounds and has the ability to agglutinate bacteria and non-self-erythrocytes. Numerous mucosal

proteins may cooperate with each other in the agglutination process in the mucus, although agglutination is detected without the addition of other proteins in the case of isolated or recombinantly generated lectins, representing that lectins can achieve agglutination and hemagglutination on their own (Kugapreethan et al. 2018). A rhamnose-binding lectin agglutinates the microsporidian (*Glugea plecoglossi*) (Watanabe et al. 2008), and another one pufflectin from puffer fish binds to the trematode *Heterobothrium okamotoi* (Tsutsui et al. 2003). Congerin from the conger eel's skin mucus causes *Vibrio angullarium* to agglutinate, but not growth suppression or bacterial death (Kamiya et al. 1988). This demonstrates that pathogen binding and growth or death inhibition are not always connected.

14.6.2 Lectins Act as a Chemotaxis

Chemotaxis in bacteria can be induced by fish mucus. Different fish stimulate chemotaxis in varied ways, and the skin, gill and gut mucus have diverse effects. Mucus from the skin and gills was more motivating than mucus from the intestine in channel catfish and skin mucus from 60% of the fish-induced chemotaxis. Different serotypes of *Vibrio anguillarum* were found to be chemotatic to skin mucus from various fishes in a research (Halberg et al. 2001). Several Vibrio strains displayed chemotactic behaviour towards mucus in gilt-head sea bream (*Sparus aurata*) in a research, which may be suppressed by high or low salinities and temperatures (Bordas et al. 1998). The chemotactic activity of *Flavobacterium column* was discovered in a research of the bacteria (Klesius et al. 2010). Sugar's capacity to block chemotactic action strongly suggests that this activity is lectin-dependent.

14.6.3 Lectins Act as an Endocytosis and Phagocytosis

Traditionally, lectins have been thought of as a component of the immune system that protects against disease; nevertheless, lectins can also aid pathogens by encouraging infections. The ability of lectins to act as opsonins, boosting pathogen phagocytic and endocytic uptake can be utilized by pathogens to gain entry to cells. The absorption of pathogens has been the subject of teleost lectin research. Many lectins have been identified to promote uptake. In comparison to the control, the F-type of lectin from sea bass (*Dicentrarchus labrax*) improved fucose inhibitable and phagocytic uptake of *E. coli* by peritoneal macrophages (Salerno et al. 2009). Galectin-1 appeared to be secreted from cells that had phagocytosis latex beads in Atlantic cod head kidney leukocytes, suggestive of that phagocytosis can encourage the making of lectin to further accelerate phagocytosis throughout infections.

14.6.4 Lectins Act as a Antimicrobial Agent

Antibiotic resistance is a critical issue, and researchers are looking for new cytotoxic compounds to combat it. Several lectins have been shown to be cytotoxic to bacteria and parasites. Still whereas lectins have been exposed to attach straight and only to cells to agglutinate, they can also behave as part of complexes when serving their biological purpose has been demonstrated in mammalian lectins. The ability to bind to infections raises the prospect of them inhibiting growth and killing them directly. Natterins are lectins with a jacalin-like carbohydrate-binding domain that binds to mannose at the N-terminus and an aerolysin-like toxin domain at the C-terminus. Natterin was isolated from the skin mucus of the Oriental stinging catfish (*Plotosus lineatus*) and Atlantic cod. The structure of the arolysin-like domain implies that it could lyse infections (Tamura et al. 2011).

Lectins also have a role in viral prevention, and the F-type lectin from rock bream (*Oplegnathus fasciatus*), RbFTL-3, which is primarily expressed in the animal intestine, can defend fathead minnow cells from haemorrhagic septicaemia virus infection in vitro (Cho et al. 2014). CsLTL-1 and lily-type lectin were up-regulated in the gills of striped murrels (*Channa striatus*) after infection with *Aeromonas hydrophila* and *Aphanomyces invadans* (Arasu et al. 2013). This implies that they play a part in the host's defence. However, because viruses, bacteria and other pathogens can take over host machinery to gain access to host cells, further research is needed to prove that an increased amount of lectin is beneficial to the host rather than being pushed by pathogens to boost infection.

14.7 Lectin from Crustaceans and Its Applications

Because invertebrates lack an adaptive immune system, they have evolved sophisticated mechanisms as part of their innate immunity. In the haemolymph and coelomic fluid of invertebrate animals, lectins can be found in practically all phyla. Several lectins have been discovered in crustaceans, with involvement in cell signalling, cell–cell contact, protein synthesis and pathogen identification. These functions are carried out due to their carbohydrate specificity. The structural and functional variety of lectins in crustacea appears to reveal distinct innate immune responses based on sugar sensitivity. Lectins, as innate response effector molecules, are involved in identification and antibacterial activity, as well as serving as opsonin in cellular defensive reactions such as encapsulation and phagocytosis. In terms of structure, the lectin molecule from crustacea is similar to the lectin from the simplest demosponge *Suberites domuncula* (Schröder and Ushijima 2003) and a complicated variable area of human IgG.

Hepatopancreas, haemolymph, haemocytes, gills, brain, heart, muscle and stomach have all been found to have crustacean lectins. The hepatopancreas has been suggested as the most critical tissue in crustacean immunity, particularly in their

humoral immune response, and it appears to be the principal site for manufacturing immune recognition molecules (Soderhall 2010). Lectins from crustacean haemolymph have been suggested as possible molecules involved in immunological recognition by acting as PRRs. Although crustacean lectins recognize a wide spectrum of glycosylated molecules, the majority of them exhibit a preference for N-acetylated carbohydrates, and all of these lectins were separated from crustacean serum using affinity chromatography (Marques and Barracco 2000). It is worth noting that several lectins have been found using molecular techniques, and their expression in many tissues has been assessed. Their specificity, however, has yet to be determined. Furthermore, it is unclear whether lectin specificity is linked to the activation of a unique immunological response in crustaceans.

Many lectins have been discovered in crustaceans to date. At the molecular and transcriptional levels, the majority of the published research discovered and sequenced lectins. However, several articles have reported on lectin protein characterization; hence, further information on the lectin–carbohydrate interaction is needed to elucidate their role in the immune system via sugar recognition. In crustaceans, lectins have a wide range of carbohydrate recognition capacities and play a role in the activation of immunological responses against a wide range of pathogens, including fungi, viruses and bacteria. There are only a few papers on the lectin–receptor interaction and how this identification stimulates the immune response in crustaceans. The cellular receptors for lectins have been discovered in haemocytes of numerous species. It is critical to figure out how lectins and their carbohydrate specificity play a role in the control of the immune response in crustaceans, as this knowledge will help the aquaculture industry prevent or cure diseases that reduce global productivity.

14.8 Conclusion

Many investigations on fish mucus lectins have been conducted, with the majority of these finding antibacterial and immunological effects. Naturally, lectins and a variety of other bioactive components found in mucus play a part in this effect. In terms of science, using the lectin alone will yield more solid results. However, because protein isolation is required, this technique is exceedingly expensive. The mucus can be composed in huge quantity from captured fish or obtained lacking injuring farmed fish. Mucus will thus be more cost efficient, although one could quarrel that a scientific explanation of the impact will be practically difficult.

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Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 15
Fish Lectins as Molecular Markers

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Abstract Lectins are a collection of primordial carbohydrate-binding protein molecules with multiple functions. These proteins exist in fish and other animals which bind to cell surface and surface glycans. Sequence and expression analysis shows they actively binds and interacts with innate and adaptative immune responses of various disease-causing agents. Immense advancement have been developed for the current existence for recreation of lectins in numerous natural processes. In this current scenario, lectins are regarded as an invaluable tool for the study of sequencing interactions among various pathogens. They also display a range of biological functions together with the development of new drugs and disease diagnostic tools for the treatment of numerous diseases. The present chapter concentrates the precise purpose of lectins sequencing and their interactions towards therapeutic implements.

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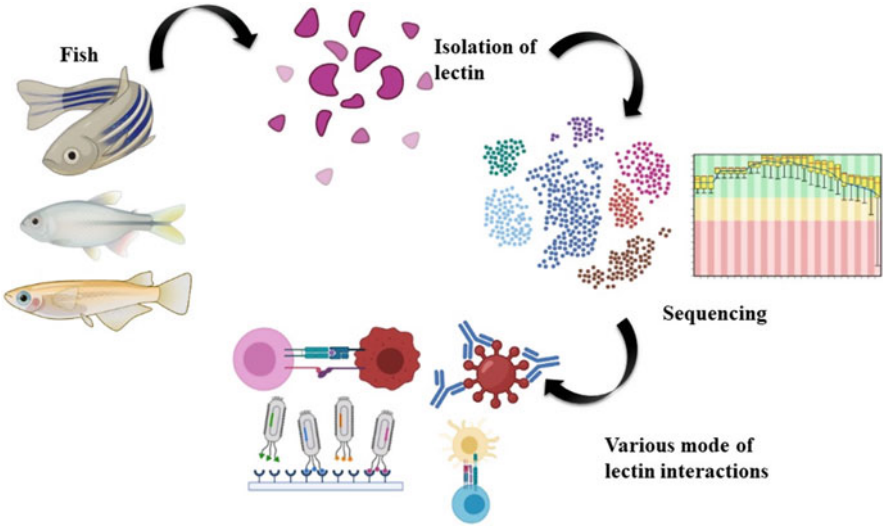
Graphical Abstract



Keywords Lectins · Sequencing · Interaction · Binding · Diagnostic tool

Abbreviations

CD209	Cluster of differentiation 209
CTLD	C-type lectin domain
C-type	Ca^{2+} dependent
DC	Dendritic cell
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DNA	Deoxyribonucleic acid
MHC	Major histocompatibility complex
mRNA	Messenger ribonucleic acid
OnBML	B-type mannose-binding lectin
RNA	Ribonucleic acid
zINTLs	Intelectin

15.1 Introduction

The carbohydrate-binding protein lectin involves many biological processes such as cell–cell interactions, glycoprotein traffics and clearance, induction of apoptosis, antibacterial and antiviral activity, mitogenic activity and antitumour activity (da Silva Lino et al. 2014). In addition, fish lectins are able to recognize carbohydrate

moieties surface receptor of pathogens and moreover induce the innate immune response in fish immune system via agglutination and immobilization of pathogens in complement-mediated opsonization of the pathogens (Elumalai et al. 2019). Furthermore, fish lectins also have some admirable properties, i.e., it involves in fertilization, embryogenesis and morphogenesis (Lino et al. 2013). Lectins have been isolated from body fluids (serum, mucus and plasma), stomach, gills, skin, oocytes, eggs and plasma of various fishes (Mistry et al. 2001; Wang and Wang 2013; Elumalai et al. 2019). Lectins are classified based on the structure, binding specificities and calcium dependency such as C-type, F-type, galectin, intelectins, rhamnose-binding lectins, I-type lectins and lily-type lectins (Ogawa et al. 2011; Arasu et al. 2017). Additionally, the trending use of lectins today paves a new way in molecular biology. Several sequences were identified and isolated from various fish species with enumerable disease-resistant properties. Lectins are renowned as key components of various scientific disciplines. Owing to their ability to interact with other molecules, it is a very useful tool to study various diseases and disorders. Lectins have potent antimicrobial properties and kill the pathogen via endocytosis, intracellular translocation of glycoproteins, binding to glycoconjugates, apoptosis processes and binding to epithelial cell of pathogens (Santos et al. 2014). In current times, lectins have appeared as the deepest biomedical kit that has been used as anti-pathogenic, immunomodulatory and anticancer agents. Molecular cloning and physicochemical characterization of the fish's lectins exposed its importance in the medical field for treating various diseases (Richards et al. 2003). This expands a novel technique in the region of molecular biology by increasing the knowledge in advanced molecular sequencing that measures deoxyribonucleic acid (DNA), protein, ribonucleic acid (RNA) and other metabolites to notice, mutation, changes in biochemical factors and diseases (Bakermans and Madsen 2002; Alwine et al. 1977). The sequencing of lectins helps to identify the biological compounds (amino acids, ligands, proteins) and their properties. Which act as a key tool for bioinformatics analysis to study the interactions of lectins with various biological molecules (protein and ligand band) through hydrogen bridges, van der Waals hydrophobic interactions and ionic interaction (Tasumi et al. 2002; Nelson and Cox 2001). Recently, unique and specific properties of fish lectin-based studies were increased. The genetic factor progression of fish lectins has been examined to illuminate their source and their existence in various tissues. Under laboratory condition, fishes are challenged in contradiction of pathogens in vivo and, hence, leads to gene expression investigation of healthy and infected animals under experimental environments (Elumalai et al. 2019). Various types of fish lectins have been used in numerous applications in medicine (antimicrobial effects, antitumour property, wound healing capacity, coagulation of blood, etc.) and in aquaculture (immunostimulant). Lectins from microorganisms play invasive role acting through immune system (Bah et al. 2013). Many lectins identified from animal tissues represent immunomodulatory effects, antiviral and anticancer properties. Lectins have a wide area of applications in medicine, pharmaceutical and food industry with the interest towards the contribution in research and technology. The exceptional characteristics of lectin proteins make them desirable for varied applications in the field of science. Lectins play a

significant role in nature that occupies a specific place in therapeutic research areas. Despite their wide functions, lectins are promising compounds that recognize specific carbohydrate structures, viz. glycoproteins, glycolipids, which regulate glycoconjugates for their physiological and pathological interactions. Lectins can be isolated from plants, animals, viruses, bacteria, fungi, algae as they recognize carbohydrates compounds makes them a promising tool in biotechnology, diagnosis and pharmaceutical, therapeutic application. Consequently, this chapter focuses on the characteristics of lectins as molecular markers exclusive in cancer diagnostics research, isolation, sequencing and interaction of lectins with various biomolecules and their therapeutic applications.

15.2 Sequencing of Fish Lectins

A broad range of functionally active lectin nucleotide polymorphisms within gene sequences lead to allelomorphic variation, and subsequently, potential phenotypes were produced. Architecturally and functionally different protein isoforms can be programmed by multiple gene families and differentially articulated in multiple tissues or by substitute pre-mRNA merging of various sequences from a single gene. Sequencing of the lectin alternatives is mandatory to increase understanding into the molecular mechanisms that yield numerous isoforms and the accurate indications that activate differential expression throughout inflammation. Separation of efficient isoforms of fish lectin is essential to elucidate whether sequence alterations deliberate modifications in carbohydrate binding, complement activation, multimeric gathering and the aptitude to fix dissimilar types of pathogenic bacteria. The specificities of lectins encompassed conceivable beneficial and therapeutic diagnostics importance from various living organisms for their existence of bioactivity, as well as testing pharmaceutical properties and medicinal plant protection. Moreover, several studies have been carried out using lectins as tools to stubborn on analysing the inappropriateness assessment of brawny neoplastic tissues.

The lectin–carbohydrate interaction has been predictable as one of the key components of innate immunity in animals. It is well understood that each type of lectins has a highly well-maintained and inflexible pattern of amino acid sequences (Lino et al. 2013). Generally, superfamily of C-type lectin (CTL) includes a large number of adherents among the animal kingdom. Ca^{+2}-dependent carbohydrate-binding of lectin mechanically involved in cell adhesion, cell communication, pathogen recognition and activation of immune responses among others (Dambuza and Brown 2015; Weis et al. 1998; Zelensky and Gready 2004). C-type lectin domain (CTLD) superfamily has been classified in 14 groups of proteins with various structure and phylogenesis (Drickamer et al. 2002). In teleost fishes, B-type lectin genes were efficaciously cloned from pufferfish (*Takifugu rubripes* and *Takifugu niphobles*) (Yuan et al. 2006; Vallejo et al. 1990), striped murrel (*Channa striatus*) (Ourth et al. 2017), turbot (*Scophthalmus maximus*) (Ren et al. 2015) and tongue sole (*Cynoglossus semilaevis*) (Ai and Xie 2005). According to previous literatures, the tissue distribution of the B-type lectins was determined

using sequencing and their abilities in uniting with bacteria and function in the antibacterial immunity were examined. Yin et al. (2019) examined the structure and function of B-type lectins from Nile tilapia and termed as OnBML whose gene was effectively cloned. Further, the mRNA expression level of OnBML was studied in different tissues of healthy fish towards bacterial (*S. agalactiae* and *A. hydrophila*) infection. Moreover, chronological patterns of *OnBML* expression in vitro after bacterial challenges were demonstrated which actively opsonize bacterial binding. Similarly, two novel C-type lectins (ToCTL1 and ToCTL2) were recognized and characterized successfully from puffer *T. obscurus* by Huang et al. (2020). The intestine and kidney lectin mRNA encoding ToCTL1 and ToCTL2 was done which upon bacterial challenges provides remarkably upregulated in vivo expression towards bacteria. Based on sequence homology of lectin, it has been described DC-SIGN genes from three species of fish, including *Fugu rubripes, Danio rerio* and *Cynoglossus semilaevis*, which were similar to mammal genes with different functional assays (Zelensky and Gready 2004). It is explained that various number of reputed gene sequences were available in salmon genome that are interpreted as DC-SIGN-like genes, in which no enthusiastic or efficient scrutinizes were involved. Moreover, while DC has not been completely secluded in *Salmo salar*, a reputed CD209 sequence was distinguished and used to illustrate a DC-like subtype of cells (Ojeda et al. 2020). Yin et al. (2019) isolated B-type mannose-binding lectin (OnBML) from *Oreochromis niloticus* with 354 bp nucleotide sequencing and 117 amino acids which shows upregulated antibacterial activity against *Streptococcus agalactiae* and *Aeromonas hydrophila*. Similarly, intelectin (zINTL) from zebrafish shows upregulated antibacterial activity against *Aeromonas salmonicida* (Lino et al. 2013). Moreover, a 1464 bp nucleotide sequencing with 281 amino acids and upregulated antibacterial activity against *Vibrio anguillarum* were sequenced form C-type lectin (PaCD209L) of *Plecoglossus altivelis* (Yang et al. 2015). Literatures on the sequencing of lectins pave a way towards cell–cell interactions, host cell–pathogens interactions and antimicrobial activity. However, future research will be innovative and concentrate towards fish lectins with developing gene sequence information against infectious agents. Moreover, various immune-related proteins were isolated from the aquatic organisms similar to fish lectins. They include lipopolysaccharide and β-1,3-glucan binding protein (Lin et al. 2008; Sivakamavalli and Vaseeharan 2013; Vaseeharan 2012); Penaeidin (Vaseeharan et al. 2012); alpha 2-macroglobulin (Lin et al. 2007; Vaseeharan et al. 2007) and a serine proteinase (Vaseeharan et al. 2006), which were isolated and cloned. These proteins fight against the aquatic pathogens and also activate the prophenoloxidase cascade.

15.3 Interactions of Fish Lectins

Fish lectins recognize (or bind) the carbohydrate structures (proteoglycans, glycoproteins, and glycolipids) of various cells, which can regulate the physiological and pathological phenomenon via host–pathogen interactions and cell–cell communications (Ogawa et al. 2011; Lino et al. 2013). Fish lectins play an active role in innate

immune response by recognizing various pathogenic microorganisms. In addition, lectins correspondingly contribute in downstream effector roles, for instance adhesion, immobilization, opsonic phagocytosis, complement initiation and augmenting the activity of natural killer cells (Vasta et al. 2011). Several previous surveys were reported regarding the role of lectins and their participation in the commencement of innate and adaptive responses of host in contradiction of potential pathogens (Elumalai et al. 2019). Different type of lectins have the potential to expand the range of carbohydrate-ligands that can be helps to recognized by endogenous and exogenous glycans. The presence of effector domains and lectin isoforms can also increase the lectin's functional efficiency and enhance its diversity in recognition of ligand (Vasta et al. 2011). Lectins are involved in the intracellular-mediated compartment process, and it recognizes the effector factors (agglutinins, opsonins, complement activating factors) and leads to recognize potential pathogens (collectins, ficolins and pentraxins) using the endogenous or exogenous ligands. (Vasta et al. 1999; Vasta and Ahmed 2008). Lectin-mediated biological processes start with cell–cell or extracellular matrix interactions, owing to lattice formed on the cell surface, and lead to activating the signalling pathways of innate and adaptive immune responses via promoting phagocytosis and activating the complement systems, modulating the development of B cells, inducing, or preventing T-cell apoptosis and processing of antigen by dendritic cells and presented for MHC of T-cell receptor (Fujita 2002; Van Kooyk and Rabinovich 2008; Jayaraman et al. 2010; Sehrawat et al. 2010). The interactions between various lectin types and the immune system contribute to the response to microbial challenge. Fish lectins are antimicrobial agent that target microbial surfaces and can kill pathogens (immobilization, opsonization and complement system-mediated pathway). They can also regulate the immune system's downstream functions by binding to antigens (Vasta et al. 2011). For example, C-type fish lectins have the potential role of anti-microbial agents (Tasumi et al. 2002; Kondo et al. 2007; Tsutsui et al. 2003). Lectin possesses mutual interactions with colony-forming potentially pathogenic microorganisms by agglutinating cells and precipitate polysaccharides, glycoprotein or glycolipids (Zhang et al. 2009, 2010). Further stimulate humoral and membrane-associated recognition molecules that may support the interaction (Vasta et al. 2011). Rhamnose-binding roe lectin isolated from chinook salmon exhibited unique antiproliferative activity against human breast cancer MCF-7 cells and hepatoma Hep G2 cells (Bah et al. 2011). Similarly, Atlantic salmon blood lectin exactly bind and interact with the mannose residues on the surface of the pathogens *Vibrio anguillarum*, the causative agents of cold water vibriosis, and *A. salmonicida* (Ewart et al. 1999). According to previous reports, galactose-binding lectin isolated from Indian catfish interacts and binds with various Gram-negative bacteria *Aeromonas* sp. Lee et al. (2016) discovered that the lily-type lectin OfLTL-2 and 3 in rock bream are articulated in the immune tissues and involved in the second phase of viral infection. Generally, lectins bind to the targeted cell surface and induce immune interaction by releasing mediators like second messengers from the cell surface (Lino et al. 2013). The ovaries of cobia provide a novel tetrameric lectin which exert antibacterial activity by generating a strong interaction towards

E. coli and cause 50% inhibition at a concentration of 250 μg (Ngai and Ng 2007). A Gb3-unique lectin unambiguously binds and interacts towards globotriose (Gb3; Gala1-4Galb1-4Glc) and is collected from catfish (*S. asotus*) egg nominated as SAL and was revealed to bind with Gb3 expressed on the surfaces of the Burkitt's lymphoma cells (Sugawara et al. 2017).

15.4 Lectin as Molecular Marker

It is important to note that many lectins have been identified by molecular techniques and that their expression has been evaluated in different tissues. Though, their specificity is still to be identified. Lectins have a wide range of amino acid sequences that are found in bacteria, viruses, animals and plants. They have a variety of activities, structures, tissue localizations and carbohydrate specificity. Although animal lectins do not have homologous primary structures, they have similar preferential binding to carbohydrates (De Schutter and Van Damme 2015). It is hypothetically possible to use lectins to progress the health of humans and animals. In addition to being exploited for analytical purposes, lectins may also be hired for therapeutic resolutions as molecular markers. The status of lectins in scientific study has been continuously rising. Lectins may be used in a diversity of applications including clinical microbiology, serology, inflammation, diet and human retort. Numerous lectins have glowed consideration owing to their budding therapeutic applications, together with anti-HIV, anti-tumour, antibacterial, anti-inflammatory and antinociceptive characteristics (Ogawa et al. 2011; Coelho et al. 2017; Sun et al. 2008). Based on their properties, lectins may be used in a diversity of applications. The antifungal, antibacterial and anti-insecticide activity of lectins might be used to switch off contagions in numerous situations. Furthermore, the formulation of anti-tumour and anti-viral drugs that are generated on lectins may be conceivable. Different biological markers are being considered in determining of a new diagnosis and management. At present, there is modest confirmation on the use of lectins as biomarkers in cell biology, biochemistry and immunology, as well as for cancer testing diagnostic purposes (Lei and Chang 2009). Figure 15.1 represents an overall mechanism of lectins as molecular markers in cancer diagnostics, their incorporation, implementation and detection of tumour in cells. Being luxurious tools in biotechnology, lectins differentiate tissues, biological fluids, carbohydrates and glycoconjugates in cells in diagnostic applications (Lam and Ng 2011; da Silva et al. 2014).

Various studies have been carried out using lectins as a biological marker on diagnosis owing to the absurdity estimation of strapping tissues. Varied apparatuses are being scrutinized in the examination of a novel accurate analysis and treatment (Ikeda et al. 2012). Lectins have also been considered for their potential ability in the recognition of ovarian cancer. Dual FDA-recognized glycoprotein biomarkers for ovarian cancer include cancer antigen 125 (CA125) and human epididymis protein 4 (HE4). Artocarpus integrifolia agglutinin (AIA), Amaranthus caudatus agglutinin

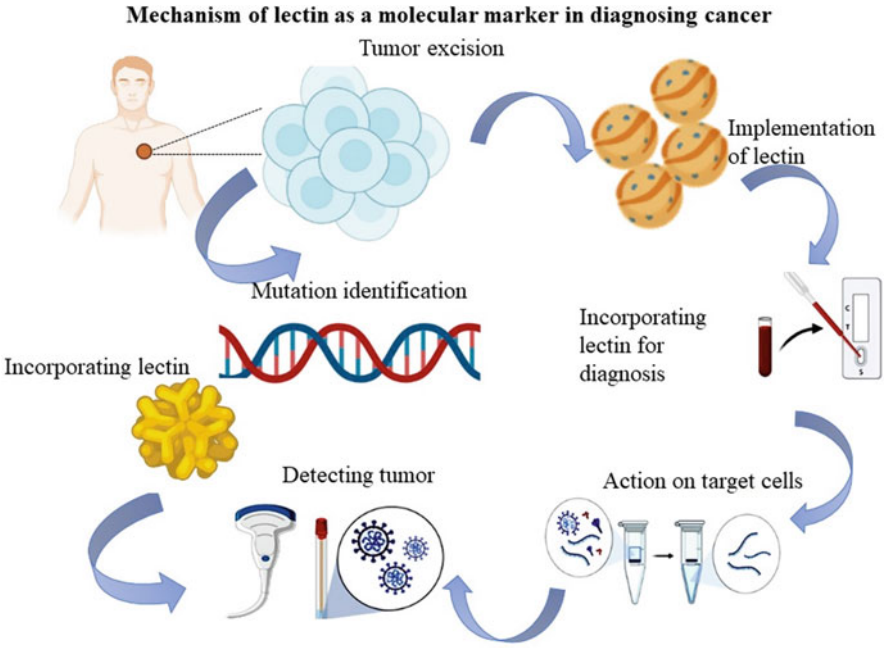


Fig. 15.1 Mechanism of lectin as a molecular marker in cancer diagnostics

(ACA), Ulex europaeus agglutinin I (UEA I) and Griffonia simplicifolia agglutinin I (GSA I) are a group of lectins (Wu et al. 2008; Chen et al. 2013). Additionally, AAL and MAA-II might be hypothetically used for the judgement of colorectal cancer as well the revise up of disease evolution. Currently, the application of lectins as a biomarker in cancer diagnosis, imaging and treatment gained a lot of contemplation among researchers (Coulibaly and Youan 2017). Moreover, lectins are used to investigate the glycan profile of transformed tissues in cancer recognition and prediction (Kannagi 2007). Lectins particularly binds to carbohydrates that permit them as diagnostic biomarkers including the MLs, ML-I, ML-II and ML-III, Gal-1,2Gal-allyl and Gal-1,3Gal-allyl. Micro lectins are habitually monoclonal proteins with diverse molecular weights, they are substantial diagnostic molecular markers in microbiology and molecular biology. At present, lectins are crudely involved in glycobiology diagnosis and plotting pathways (Annuk et al. 2001). Though, advanced investigation in drug delivery system was used to magnify the probabilities for medical transformation. Numerous lectins were used as potential biomarkers for primary detection of malignant or else as persuader of autophagy. Detectably, galectin-1 has an extensive parade reliant on the cell type, background and convenience of counterreceptor for expressing glycosylation and protein. Preferably, investigation on lectin originated previously two eras ago (Smetana et al. 2013). Using lectins as a molecular marker helps in the identification of glycoconjugates with anomalous glycan configuration expressed by cancer cells

(Kjeldsen et al. 1988). Moreover, lectins detect non-immune proteins or glycoproteins with definite penchant for the carbohydrate mediety of glycoconjugates (Goldstein et al. 1980). Generally, fish lectins have been broadly used as a biomarker for determining tissue cells-associated host defensive functions (branchial epithelium, renal interstitium, hepatic sinusoidal, intestinal sub-mucosal granular layer, skin and particularly within circulating granulocytes) (de Santana Evangelista et al. 2009). Hence, lectins lean towards an auspicious therapeutic and diagnostic biological marker.

15.5 Therapeutic Applications of Lectins

Many of the researchers reported that lectins have healing properties based on their origin. Very limited studies are available in fish lectin's healing activity. But the major study was focused on plant lectins. For example, Cramoll-1,4 lectin isolated from seeds of *Cratylia mollis* Mart, and it is able to treat the cutaneous wounds in mice (de Melo et al. 2011). Similarly, Artin M lectin extracted from seeds of *Artocarpus heterophyllus* have properties of healing the wound of palatal mucosa of dog. This Artin M induces the proliferation of fibroblasts cells in healing area (Kim et al. 2020). Moreover, lectin from red algae *Amansia multifida* have 30 kDa β-strand elements, and it has altered the inflammatory parameters and reduced the formation of oedema which shows their anti-inflammation property (Mesquita et al. 2021). Marine red algae *Bryothamnion seaforthii*-derived lectins have pro-healing effects via activating the fibroblasts, migration of polymorphonuclear cells in mice's skin wounds (Gonzaga do Nascimento-Neto et al. 2012). Coriolano et al. (2014) reported that Parkia pendula seed lectin has healing cutaneous wounds in both normal and immunocompromised mice. Moreover, fish lectin acts as antimicrobial agent and also controls the pathogenic infection, meanwhile performance as immunomodulator. Lectin from haemolymph of *Metapenaeus dobsoni* (68 kDa) has bacterial agglutination activity against *Vibrio parahaemolyticus* and *Aeromonas hydrophila* (Rubeena and Preetham 2019). Likewise Rubeena et al. (2019) C-type lectin (75 kDa) from *Etroplus suratensis* has antibacterial activity and also antibiofilm activity of bacterial pathogens. *Colossoma macropomum* serum-derived lectin has been recognized by D-fucose, carbohydrate D-galactose and 1-*O*-methyl-D-galactopyranoside, and it has antimicrobial activity against pisciculture bacterial pathogen (Carvalho et al. 2012). Other than fish lectin, plant lectin also has great antimicrobial properties. For example, *Phthirusa pyrifolia* leaf lectin (15.6 kDa) has antimicrobial activity against Gram-positive *Staphylococcus epidermidis*, *Streptococcus faecalis* and *Bacillus subtilis* and Gram-negative *Klebsiella pneumonia* and also has antifungal activity against *Fusarium lateritium* and *Rhizoctonia solani* (Costa et al. 2010). In addition, lectin has great affinity to carbohydrate binding domain, which leads to lectin used in a variety of applications. Generally, lectins have agglutination activity, toxic to the pathogen, stimulate the immune-related pathways, mitogenic stimulation, stimulate the apoptotic pathways, etc. For that,

recently lectins are used as disease diagnostic tools. For example, from clinical serum samples, detection of dengue glycoprotein by *Cratylia mollis* lectin-based composite acts as biosensor (Avelino et al. 2014). Recently, lectin acts as a histochemical marker for detecting disorders like cancer (Dwek et al. 2001; Beltrão et al. 2003) and pathogens (Leal et al. 2012). Lectins employed to examine the glycan profile in transformed tissues establish beneficial tools for diagnosis and prognosis of cancer.

15.6 Future Direction

Many researches perform the isolation of lectin molecules from fish species. The literatures only elucidate their biological effects such as anti-microbial efficacy, anti-cancer efficacy, immunomodulator, etc. In this situation, it is essential to find out the amino acids that are arrayed in the peptide chain of the lectin molecules and their length (base pair). It also aids to determine the structure and function and helps to understand their cellular interactions. Only very limited studies are available in sequencing of fish lectins (Yin et al. 2019; Lino et al. 2013; Yang et al. 2015). Moreover, further research should focus on sequencing of fish lectins and its molecular mechanisms for the cellular process and disease prevention for aquaculture.

15.7 Conclusion

This chapter sums up the lectins as molecular makers and isolation, sequencing of lectin as tools to memorize the interactions in disease management. The exceptional characteristics of lectins were noticeable and further search on the therapeutic, pharmacological properties should be dealt in detail to improvise the utilization of lectin in various other commercial sector. Documentation of efficient properties, predominantly those concerning gene sequence information, was used relatively and well characterized for infectious agents. It concludes that lectins have a great potential to be used as antipathogenic agents as they actively interact and bind as investigated in previous literatures. The biological material's lectin-based therapeutic agent and diagnostic tools will be mostly used in medical and research field in near future.

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Part VI
Modern Trends/Advancements in Lectin Research

Chapter 16
Application of Fish Lectin in Human and Veterinary Medicine

Mani Divya and Baskaralingam Vaseeharan

Abstract Mucus is encased in the membranes of several fish species. These mucus build-ups serve as a barrier between the fish and the environment. It is essential to remember that mucus is rich in lectins and has a function in disease defense. Lectins are carbohydrate-binding proteins produced by a variety of species. Lectins are simple molecules with a long list of well-known uses. Lectins are a kind of protein found all around the world that uses its binding sites to selectively differentiate between glycoconjugates. Structure, carbohydrate specificity, and species location are used to classify lectins. Lectins are a structurally diverse group of proteins that are distinguished by their capacity to preferentially bind carbohydrate moieties of cell surface glycoproteins. Plant-, microbial-, and animal-derived lectins may be membrane-bound. Tetramers with the same subunits make up lectins. Muscle proteins are abundant in fish frames that have been intuitively deboned. As a result, dispensation by-products-derived fish proteins may be digested to recover protein biomass. The amino acid content of these muscle proteins makes them nutritionally valuable and appealing. The usefulness of vertebrate lectins in innate immunity is now being explored, although the results are mostly unrealized for fish. Recent study, as well as the critical function of routes and applications cited in a variety of sources, has aided in describing how these chemicals activate fish's defensive systems. The aim of this chapter is to look at the use and usage of fish lectin in veterinary medicine and human medicine. Many investigations have shown that fish lectins exist. Some of these investigations are described and tabulated in this chapter, along with fish lectins discovered since the previous major review. In conclusion, careful lectins are described in more detail for practical use. To summarize, this chapter gives an overview of fish lectin research, with a focus on their application and applications in veterinary medicine, as well as their performance and prospective characteristics.

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Abbreviations

ComaSeL	*Colossoma macropomum*
CRD	Carbohydrate recognition domain
DlFBL	*Dicentrarchus labrax* fucose-binding lectin
Dln1	*Danio rerio* primer
HIV	Human immunodeficiency virus

16.1 Introduction

Lectins are carbohydrate-binding proteins that are found in almost all living species. They are involved in immunological responses as well as other biological activities, including the breakdown of carbohydrates (Drickamer 1999). Lectins are proteins that are distinguished by their capacity to bind carbohydrates to other proteins (Nilsson 2007). Non-immune origin with widespread distribution in nature that identifies and reversibly binds to carbohydrates and glycoconjugates and that are either free or attached to cell surfaces via particular binding sites (Correia et al. 2008). Furthermore, they are involved in a variety of biological activities (Cooper et al. 1994), including innate and adaptive immunological responses (Vasta and Ahmed 2008; Dara et al. 2005). Watzi et al. (2008) discovered that lectins are selective for monosaccharides or small oligosaccharides, glycoproteins, and polysaccharides (Thakur et al. 2007). The presence of a carbohydrate recognition domain (CRD) in the crystal structure of lectins distinguishes them from one another. In terms of sugar specificity, lectins are divided into many families and groupings depending on their CRD (Sanchez-Salgado et al. 2017). The architectures of lectins are critical for defining the characteristics of glycan classes that have been identified in a variety of animals. When it comes to animals, lectins are divided into various groups based on their common evolutionary background or the similarity of their structural folds (Russell and Lumsden 2005). The biological significance of lectins is still up in the air. Lectins may have a role in the transit of sugar or the storage of carbohydrates. Some of the lectins may be involved in the binding of symbiotic rhizobia to create root nodules, according to the research (Kilpatrick et al. 1984).

The presence of lectins in the hemolymph and coelomic fluid of invertebrate creatures may be found in almost all phyla of the animals. Lectins have been identified and described from a variety of vertebrate species, including fish (Carvalho et al. 2012), snakes (Nunes et al. 2012; Aranda-Souza et al. 2014), and other animals (Nunes et al. 2012; Aranda-Souza et al. 2014). In humans, in example, many well-characterized lectins are found in a variety of organs and cells, including the lungs and kidneys (Kishore et al. 2006; Sorensen et al. 2007). The isolation of lectins from marine resources, particularly from fish, has grown significantly during

the past several decades. Fish lectins have a wide range of structural and functional properties, including carbohydrate specificities that are unique to each individual fish species. Fish lectins are concerned about the requirement for strong evidence to support the significant involvement of plasma in the inborn defense of fish (Yano 1996; Russell and Lumsden 2005).

In fish, there is a great deal of variation in the production and distribution of lectins, which suggests that they have different roles in innate immunity at different stages of development, from the moment oocytes are released and fertilized until adulthood (Vasta et al. 2011). Using the exterior mucus of the *Genypterus blacodes*, scientists discovered the first fish lectin ever isolated (Oda et al. 1984). Recently, numerous lectins were isolated from various organs and tissues, including the stomach, gastrointestinal tract (gut), liver, gills (eggs), skin (mucus), serum, and plasma of different fish families, as well as many others (Whyte 2007; Ng et al. 2015). As a result of the interaction between microorganisms and the mucus on the surface of the fish skin, lectins, agglutinins, lysozymes, lysins, and immunoglobulins are found in the mucus of the fish skin (Benhamed et al. 2014).

Bacteria, viruses, fungi, and parasites are all recognized and agglutinated by lectins from a variety of fish families. The number of research on different lectins, including their discovery, isolation, and physiochemical characterization, is growing at an exponential rate. Currently, the majority of functional characteristics, particularly those lectins with downstream effects, have not been identified, and, more significantly, the relevance of these features in clinically relevant fish illness has not been validated. Lectins are unique in the field of biotechnology because of the many applications and other prospective uses that are being examined and researched on a daily basis by researchers in the field of lectinology.

As important tools in research spanning a wide range of fields of science, lectins and their characteristic properties, which are primarily derived from their ability to bind glycoconjugates, stand out as particularly useful in areas such as biochemistry, cellular and molecular biology, immunology, pharmacology, medicine, and clinical analysis (Araújo et al. 2013; da Silva et al. 2015; Santos et al. 2014). In a similar way to humans, the fish immune system is divided into two types: innate and adaptive. Both are engaged in cell-mediated protection as well as the production of humoral components. Because fishes are continuously exposed to a wide variety of harmful microorganisms that may be found in the aquatic environment, the lectins found in their skin mucus serve as an essential barrier.

Aside from that, fish lectins serve an important role in many biological systems. Lectins as bioactive molecule acting on human tumor cells has led to the potential of their future usage in biotechnological applications gained through research into these lectins (Lam and Ng 2011). Innate immunity, which is based on pattern recognition, serves as the first line of defense against infections, and pattern recognition receptors are capable of identifying distinct microbial patterns that are shared by closely related bacteria, allowing them to be eliminated (McGreal et al. 2004; Jang et al. 2015; Li et al. 2017). In other vertebrates, such as mammals, the significance and functions of innate immune components such as circulating lectins have long been known, as has been the case in humans (Fock et al. 2001).

Abundance of aquatic life even in eurythermal fish, acquired immunity is reduced at low temperatures; nevertheless, some studies suggest that certain components of the fish's innate immune system may be less affected by temperature fluctuations than others (Ewart et al. 2001). Lectins and other innate immune effectors may be much more important in fish than in mammals when it comes to the acquired immune response since fish differ from mammals. Numerous studies have examined how salmon's immune system responds when exposed to lectins. If these findings could be extended to the innate immune system's components, it seems that increasing innate immunity would be the most effective way to increase disease resistance in the fish population.

16.2 Fish Lectin

The study of fish lectins has exploded in the last few decades due to a growing interest in the proteins' role in vertebrates' innate immune system. Lectins are proteins that may be found both within and outside of cells and are involved in the breakdown of proteins. Several lectins with potential immunological activities have been identified from mucosal surfaces of fish, indicating that the mucosa plays an essential role in defense against pathogens (Brinchmann et al. 2018). Aside from that, lectins from fish play an essential function in a variety of biological systems. The presence of fish lectins in tissues and cells associated with host defensive functions (renal interstitium, branchial epithelium, hepatic sinusoidal, intestinal granular layer, skin, and circulating granulocytes) has been well documented; however, only one mannose-binding B-type lectin, isolated from the mucus and sting of the venomous fish *Scorpaena plumieri,* has been identified. Lectins of the following kinds are often found in fish: C-type lectins, F-type lectins, galectins, lectins that bind to the sugar rhamnose, ricin, lily, and 6x-propeller/tectonin (de Santana Evangelista et al. 2009; Lopes-Ferreira et al. 2011).

There are a number of non-immunological tasks that they conduct in addition to immune-related ones including pathogen identification and agglutination as well as opsonization and activation of the complement system and phagocytosis (Ng et al. 2015; Vasta et al. 2011). Salmon serum lectin included the first lectin characterized in terms of structure and immunological action. A study was done in the following years to isolate mannose-binding lectins from four different species of fish: sea lamprey, common carp, and turbot (Jackson et al. 2007; Nakao et al. 2006; Ourth et al. 2008; Zhang et al. 2010). Fish-derived lectin and its application are utilized for human and veterinary purposes (Fig. 16.1). The lectins derived from animals were classified into three categories based on CRD comparison. This constant and unchanging sequence of amino acid residues is seen in all lectins, regardless of the lectin type they are found in (Ewart et al. 2001; Loris 2002; Suzuki et al. 2003). A substantial rise in fish lectin function research utilizing immunological and molecular biology techniques has been seen in recent years (Bah et al. 2011).

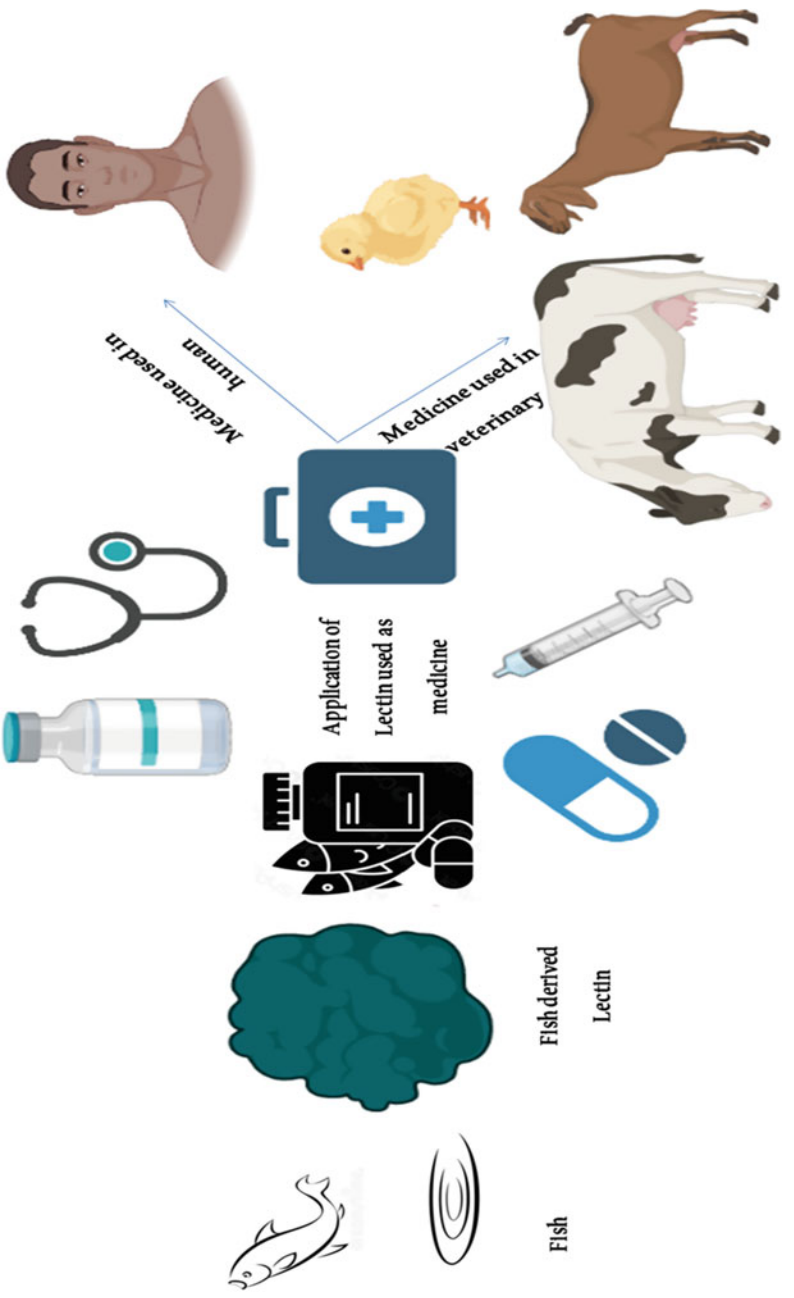

Fig. 16.1 Fish lectin used for human and veterinary medicine

Apart from that, fish lectins are involved in a variety of other processes, including agglutination, fertilization, immobilization of pathogens via complement-mediated opsonization, and death of pathogens (Dong et al. 2004; Russell and Lumsden 2005). These results show that fish mannose-binding lectins bind pathogens and play a role in the innate immunity lectin complement cascade, which is part of the lectin complement system. Serum mannose-binding lectins (Zhang et al. 2012) and blue catfish (*Ictalurus furcatus*) have been identified and their binding activity has been described in channel catfish (*Ictalurus punctatus*) and blue catfish American aquaculture relies heavily on catfish, which is why it is so popular throughout the world. Mannose-binding lectin levels in catfish have been related to the fish's resistance to infection with *Edwardsiella ictaluri*, according to the research (Ourth et al. 2007).

Fish lectins may still be released into the extracellular compartment, where they can end up on the cell surface or as soluble components in biological fluids, depending on the species (Vasta et al. 2011). Despite the fact that the general lack of functional information has not altered much during the intervening time, there are several notable exceptions. Inflammatory and immune-mediated defense lectins (lectins that engage in immunity or inflammation) are found on phagocytes, in plasma, and on mucosal surfaces and have a wide carbohydrate specificity as well as the capacity to bind to the surfaces of a variety of pathogenic pathogens. Because many lectins have been reasonably well researched, they are generally considered to be soluble pattern recognition receptors and to be a critical component of the innate immune system (Russell and Lumsden 2005).

16.3 Fish Lectin Used in Human and Veterinary Medicine

It is theoretically feasible to utilize lectins to improve the health of humans and animals. In addition to being utilized for diagnostic purposes, lectins may also be employed for therapeutic purposes. The importance of lectins in scientific study has been continuously growing. Lectins may be used in a variety of applications including clinical microbiology, serology, inflammation, diet, and human response.

In addition to being unique and naturally occurring cytochemical and histochemical tools, lectins have the potential to serve as diagnostic reagents for clinical microbiologists, which may further highlight their significance as prospective diagnostic reagents (Kumar et al. 2012). It is important to remember that protein–carbohydrate interactions are an important part of the host defense mechanism. A substantial number of immunological processes are mediated by glycobiological interactions, according to a large body of research (Gleeson 2008; Dam and Brewer 2010; Rabinovich et al. 2012).

Because carbohydrate structures such as glycoproteins and glycolipids have been shown to be involved in a variety of physiological and pathological functions, including host–pathogen interactions and cell–cell communication in a variety of

cell types, lectins are one of the most promising candidates for drug discovery from natural resources (Bonazzi and Cossart 2011; Ogawa et al. 2011; Rabinovich et al. 2012). Several lectins have sparked attention due to their potential therapeutic applications, including anti-HIV, anti-tumor, antibacterial, anti-inflammatory, and antinociceptive characteristics, to name a few (Ogawa et al. 2011; Coelho et al. 2017; Sun et al. 2008). A number of physiological conditions, such as the beginning of disease, alter the pattern of glycosylation of cellular proteins. As a result, any alteration in the glycan portion of a particular glycoprotein may be indicative of the disease that produced the change in the first place.

Studies of lectins and carbohydrates are at the forefront of research in order to block, activate, or exploit protein–carbohydrate interactions as a consequence of significant advancements; especially in medicinal chemistry, studies of lectins and carbohydrates are at the forefront of research (Hudak and Bertozzi 2014; Sharon 2008). In recent decades, lectins have been identified in a wide range of marine fish species, with potential medicinal use. Because lectins act as surface markers for cytotoxicity, cell adhesion, and tumor cell identification, they may be used in the diagnosis and treatment of cancer (Ghazarian et al. 2011; Mody et al. 1995). Lectins, which bind to viruses, may be utilized as antiviral drugs in the future. Dln1, a natterin-like protein produced from zebrafish, has been suggested as a possible antiviral medication because it has a high affinity for the gp120 antigen of HIV (human immunodeficiency virus) (Jia et al. 2016).

Cell surface lectins that inhibit viruses from attaching to cell surfaces have been found to reduce viral replication, but care must be taken to ensure that the lectins utilized are not harmful. Dln1 also contains an aerolysin-like component in addition to the mannan-binding lectin section, which might be problematic if it were used to lyse human cells since it could induce pore development (Jia et al. 2016). The ability to lyse cells, on the other hand, may be useful in the treatment of cancer, as well as in the targeting of viruses and parasites in certain circumstances.

If the lectin component of the molecule is altered, DLn1 and other fish natterins (Patel and Brinchmann 2017) may be able to show selectivity for pathogens/parasites or cancer cells. This may open the door for medicinal uses in the future. A study of the European bass's fucose-binding lectin (*Dicentrarchus labrax*) revealed proof of concept that teleost lectins may destroy cancer cells in humans (DlFBL). Adenovirus delivery of this lectin causes apoptosis in human cells, including Hep3B and BEL-7404 hepatocellular carcinoma cell lines, A549 lung cancer cell line, and SW480 colorectal carcinoma cell line (Wu et al. 2014a, b). When human cancer cells are treated with adenovirus-mediated delivery of Japanese eel lectin 1, the cells undergo apoptosis (Li et al. 2016). According to both studies, the PRMT5-E2F-1 pathway is responsible for the lectin-induced apoptotic stimulation. Lectins produced from the skin of the Japanese eel, which may be given directly to the cells, can kill human K562 cells. This indicates that lectins may be utilized to directly destroy cancer cells (Kwak et al. 2015).

The galectin AJL-1 from the Japanese eel possesses biofilm inhibition capacity, according to Takayama et al. (2009), and its application in the treatment of

periodontitis is suggested. With the increase in drug resistance, it is becoming increasingly important to create novel chemicals that can interact with bacteria, yeast, and parasites to stop them from growing or killing them. Fish are an attractive target for bio prospecting in the quest for bioactive chemicals since they are by far the most abundant group of vertebrates, with over 30,000 known species. According to certain reports, many types of lectins have antibacterial action against veterinary and/or human infections. However, more investigation into this field is required (Brinchmann et al. 2018).

16.4 Application of Fish Lectin

Depending on their characteristics, lectins may be used in a variety of applications. The antifungal and anti-insect properties of lectins may be used to control infections in a number of different situations. In addition, the development of antitumor and antiviral medicines that are based on lectins may be possible. Lectins are now the focus of much research. In addition to being utilized for diagnostic purposes, lectins may also be employed for therapeutic purposes (Table 16.1). Clinical trials may be of assistance to researchers in their investigations of the therapeutic effects and toxicity of lectins in humans. Also worthwhile is determining whether lectins have synergistic effects with existing antimicrobial agents, which may not only contribute to greater understanding of antimicrobial mechanisms, but may also have applications from a clinical standpoint as well as from the standpoint of pharmaceutical development (Kumar et al. 2012).

Lectins are used in a variety of applications that may be classified as follows:

1. The presence of lectins in tumor markers.
2. Lectins, odontogenic cysts, and malignancies of the mouth.
3. Lectins in clinical microbiology, third edition.
4. The use of lectins in serology.
5. The presence of lectins in food and the response of humans.
6. The role of lectins in inflammation.

Lectins from a number of fish families are capable of recognizing and agglutinating a wide range of pathogens, including bacteria, viruses, fungus, and parasites. Because of the rapid onset of the acute phase response and the wide range of microbial species that have been identified, lectins clearly provide an advantage over other immune repertoires in that they provide an immediate opportunity to eradicate pathogens before the other immune repertoires are activated.

In addition to their capacity to bind and agglutinate pathogens, lectins have the ability to activate the complement system, indicating that they are endowed with not just recognition but also effector capabilities. In experimental animals, many lectins have been found to exhibit immunomodulatory effects, including mitogenic activity (Ngai and Ng 2007). Furthermore, the binding of lectin to the target cell surface may result in the release of mediators from the cell surface, such as second messengers,

Table 16.1 Lectin derived from several types of fish sources and its applications

S. no	Fish sources	Type of protein	Effect and applications	References
1.	*Nemopilema nomurai*	Lectin	cDNA cloning of a jellyfish lectin	Imamichi and Yokoyama (2010)
2.	*Aristichthys Nobilis*	Lectin	Agglutination and inhibition of pathogen growth	Pan et al. (2010)
3.	*Etroplus suratensis*	C-type	Agglutination and interfering with biofilm formation	Rubeena et al. (2019)
4.	*Oreochromis niloticus*	C-type	Agglutination and binding activity	Mu et al. (2017)
5.	*Anguilla japonica*	C-type	Agglutination	Tasumi et al. (2002)
6.	*Channa striata*	A peptide (QP13) from a Lily-type lectin	Antimicrobial activity	Arasu et al. (2017)
7.	*Salmo salar*	C-type	Binding activity	Ewart et al. (1999)
8.	*Colossoma macropomum*	ComaSeL (serum lectin)	Antimicrobial activity	Maciel Carvalho et al. (2012)
9.	Yellow croaker	Galectin	Functional analysis	Zhang et al. (2016)
10.	Rock bream	Fish-egg lectins	Enhanced antimicrobial activity on pathogens	Kim et al. (2011)
11.	Japanese bullhead shark	C-type lectins	Blood coagulation binds bacteria	Tsutsui et al. (2015)
12.	Cobia fish	C-type lectins	Mitogenic activity	Ngai and Ng (2007)

which may result in an immunological response (Coriolano et al. 2012; Lino et al. 2013). Lectins produced from a number of sources exert cytotoxic effects on many types of cancer cells, including the activation of cell death pathways and the inhibition of cell growth (Coelho et al. 2017). It has been found via study on fungal lectins that they are very effective modulators of the immune response.

According to a recent study by Lv et al. (2016), lectins from various sources.

1. Are used as immunomodulators and biomarkers in both vivo and in vitro,
2. Assist in the induction of mitosis, and
3. Have a role in the development of infection and inflammation.

With the use of immobilized Concanavalin A, glycopeptides with biantennary and hybrid N-linked structures, as well as high-mannose glycans, which are abundant in both embryonic stem cells and embryoid body phases of the embryonic development, were isolated (Alvarez-Manilla et al. 2010). The carbohydrate specificity of lectins is used to detect and differentiate between samples. The digoxigenin-conjugated lectins conjugated to steroid haptens enable the immunological identification of the structures of the attached lectins. Because lectins that

specifically identify terminal sugars are used, it is possible to tell where a carbohydrate chain starts and terminates. It was used to change the glycosylation patterns of cell surface glycoconjugates during the thymocyte selection process in mice during the postnatal period, and the findings were encouraging (Balcan et al. 2008).

16.5 Conclusion

The importance of lectins in scientific study has gradually increased throughout time. This may be due to their general capacity to bond with particular glycoconjugates, which may explain their popularity. Lectins of animal origin have been successfully utilized with a variety of microorganisms to link virulence with the surface characteristics of the microbe. It is possible that these varied biological products may be utilized as immunostimulators or immunological amplifiers in fish and those they could have novel uses in medical therapy and diagnostics. Although the emerging biotechnology sector is starting to provide these fish lectin goods, they are presently only available at a high cost because to the high cost of production. Lectins are capable of distinguishing between different strains of bacteria and other pathogenic agents. Empirical testing methods may be used to show the use of lectins in clinical microbiology. It is important to note that lectins are one-of-a-kind naturally occurring cytochemical and histochemical instruments, which may serve to further highlight their potential therapeutic and medicinal applications in both human and veterinary medicine. Fish lectins have been identified, and additional research is being done on their biological activities and methods of action. By improving the synthesis of lectins, it is possible to discover new uses for lectins.

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Chapter 17
Molecular Cloning and CRISPR Techniques in Fish Lectin Research

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Abstract Lectins are carbohydrate-binding proteins found in viruses, prokaryotes, and eukaryotes. Lectins are associated with numerous biological capacities like cell attachment, phagocytosis, complement activation, and innate immunity. Fish lectins play a significant part in treatment, embryogenesis, and morphogenesis. There are many different types of fish lectins, and they are classified based on their structure, function, namely galectins, C-type, Pentraxins, Calnexins, I-type, F-type, and L-rhamnose lectins. The molecular cloning of different fish lectin-associated genes and its promoter regions has been cloned over past years. The cloning of cDNA sequences by techniques including RACE-PCR yields full-length cDNA. Also, for the cloning of fish lectins, gene-specific primer pairs were developed which was based on the expressed sequence tag (EST) acquired from random sequencing of a cDNA library utilizing fish liver and other organs. Fish lectins were successfully cloned, and its potential function in fish immunology was also investigated according to the literature. The CRISPR/Cas9 system is a strong, effective tool for editing genomic DNA sequences, including gene knockout in fish, using RNA-guided site-specific DNA cleavage. The efficacy of CRISPR/Cas9 knockdown via microinjection was demonstrated utilizing two genes in fish lectins such as TICAM-1 and RBL. CRISPR technology was found to be an efficient way to expedite genetic improvement of farmed aquatic fishes and can be depicted as a promising strategy for the efficiency, precision, and predictability in aquaculture research. These techniques might contribute to a better understanding of the specific fish lectins involved in host–pathogen relationship contributing to fish innate immunity and can aid in exploring novel lectins or cloning procedures for aquaculture development.

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Abbreviations

BLAST	Basic local alignment tool
cDNA	Complementary DNA
CRD	Carbohydrate recognition domain
CRISPR	Clustered regularly interspaced short palindromic repeats
CRP	C-reactive protein
CTLD	C-type domain-like domains
CTLs	C-type lectin
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EPN	Glu-Pro Asn
ER	Endoplasmic reticulum
EST	Expressed sequence tag
FTLD	F-type lectin domain
Gb3	Globotriaosylceramide
GE	Gene editing
gRNA	Guide RNA
IFN-c	Interferon gamma
IL	Interleukin
IL-6	Interleukin 6
kDa	Kilodalton
LPS	Lipopolysaccharide
LrGal-9	*L. rohita* galectin-9
MBPs	Mannose-binding proteins
mRNA	Messenger RNA
NCBI	National Center for Biotechnology Information
ORF	Open reading frame
PAMPs	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
PRRs	Pattern-recognition receptors
PTX3	Pentraxin 3
QPD	Gln-Pro Asp
RACE	Rapid amplification of cDNA ends
RBL	Rhamnose-binding lectin
RNA	Ribonucleic acid
SAP	Serum amyloid P
SHL	Snakehead rhamnose-binding lectin
SMART	Switching mechanism at 5′ end of RNA transcript
TICAM1	Toll/interleukin 1 receptor domain-containing adapter molecule
TLR	Toll-like receptor

UTR	Untranslated region
WND	TrpAsnAsp
XTLs	X-type lectins

17.1 Introduction

Lectins are carbohydrate-, proteoglycan-, glycolipid-, and glycoprotein-binding proteins. Due to these features, lectins can be used in a variety of biological processes, including cell–cell interactions, apoptosis, antibacterial, and antiviral activity as well as cancer cell proliferation inhibitory and antitumor inhibitory capabilities (Gabor et al. 2004). For the purpose of developing new medications, lectins have been extensively studied in marine bio resources for their different medicinal properties. Viruses, bacteria, cyanobacteria, and yeast, as well as plants and animals, contain these proteins. Some fish lectins may be found in intracellular compartments influencing certain processes such as RNA splicing, protein folding, and protein trafficking (Vasta et al. 2011; Hébert 2000). And it was found that fish lectins can still be released into the extracellular compartment, with two possible destinations: cell surface or soluble components in biological fluids.

In biological fluids like serum and mucus, lectins can be present both intracellularly and extracellularly. Several lectins with suspected immunological functions have been identified from mucosal surfaces of fish, implying that the mucosa plays an essential role in defense. Lectins are proteins that have the ability to attach carbohydrates such as mannose, galactose, lactose, *N*-acetyl glucosamine, *N*-acetyl, fucose, and rhamnose with substantial specificity in the presence or absence of divalent cations. They function as receptors that recognize carbohydrate molecules on pathogen surfaces and bind to them specifically (Nilsson 2007). There are three primary infection pathways in fish: the gastrointestinal system, the gills, and the skin. As fish comes in contact with water and the harmful germs along with, their skin serves as a vital immune structure serving as a protective armour (Ángeles Esteban and Cerezuela 2015). The presence of lectins in skin mucus are reported, and further research has given an overview of proteins, nucleic acids, carbohydrates, and lipids present in skin mucus and claimed that proteins or antimicrobial peptides such as lectins to have immunologically important activities (Wang et al. 2013). Lectins have been found in a variety of fish tissues, including those of the Japanese eel, conger eel, electric eel, bighead carp, gibel carp, grass carp, perch, powan, zebrafish, toxic moray, cobia fish, steelhead trout, Japanese trout, Atlantic salmon, Chinook salmon, olive rainbow smelt, rainbow smelt, Arabian Gulf catfish, channel catfish, etc. The lectins were isolated from gills, eggs, electric organ, stomach, intestine, and the liver. Skin, mucus serum and plasma have also been found to contain lectins. The molecular weight, number of subunits, glycosylation, sugar binding selectivity, and amino acid sequence of the lectins vary. Antimicrobial, anticancer,

immunoregulatory, and developmental actions are among their functional responsibilities (Singh et al. 2014).

Lectins are classified into distinct groups based on their structure, binding specificities, and calcium dependence, such as C-type lectins, F-type lectins, galectins, intelectins, rhamnose binding lectins, I-type lectins, Lily-type lectins, and so on. Among them, Ficolls, galectins, calnexin, pentraxin, F-type lectins, intelectins, and mannose-binding proteins (MBPs) are recognized to play key roles in the innate immunity and disease resistance in aquatic organisms, including both cartilaginous and bony fish (Vasta et al. 2017). Fish serum, skin mucus, and other tissue have been found to include members of most lectin families known in mammals, including CTLs, XTLs (X-type lectins), and galectins (Vasta et al. 2011). C-type lectins, F-type lectins, galectins, rhamnose-binding lectins, ricin-type, lily-type are the most common types of fish lectins which are more significant in terms of molecular cloning and genome editing aspects (Ogawa et al. 2011).

The collection of experimental procedures used to produce a population of organisms containing the same molecule of recombinant DNA is known as molecular cloning. This is constructed in vitro before being transferred to a host organism that can control its reproduction in tandem with its growth. This is generally done with an easy-to-grow, nonpathogenic *Escherichia coli* laboratory bacterium strain (Rehse et al. 2009). A single modified *E. coli* cell containing the required recombinant DNA may simply be grown exponentially to produce nearly infinite identical copies of the DNA. As a result, molecular cloning may be thought of as an 'in vivo Polymerase Chain Reaction (PCR)' that isolates and expands a desired fragment of DNA. Molecular cloning, on the other hand, provides more flexibility, higher fidelity, larger yields, and lower cost than PCR. The discovery of bacterial enzymes known as 'restriction endonucleases', which break DNA molecules at particular places determined by their sequence, paved way for the development of molecular cloning techniques (Shankar et al. 2017). Researchers can use restriction endonucleases to break up big DNA fragments into smaller bits, which are then linked together with other DNA molecules (vectors) using a DNA ligase enzyme. Plasmids, which are tiny circular DNA molecules physically separate from chromosomal DNA and capable of autonomous replication, are the most often employed vectors. Restriction endonucleases produce either 'sticky ends', which have a single-stranded overhang (either on the 3′ or 5′ ends), or 'blunt ends', which have no overhang. Both of these sorts of ends may be ligated together, and each has its own set of benefits and drawbacks. The two overhangs to be linked must contain complimentary Watson–Crick base pairing for a sticky-end fragment ligation to be effective (Chen et al. 1989). A blunt-end ligation, on the other hand, does not need this, making it considerably more versatile. Due to a lack of binding stability between the two fragments, blunt-end ligation is significantly less effective than sticky-end ligation. Importantly, sticky ends may be changed to blunt ends enzymatically (either by 'filling in' missing nucleotides or eliminating overhangs), and vice versa (by employing 5′-3′ or 3′-5′ exonucleases to produce new overhangs) (Bertero et al. 2017). 'Traditional' (or conventional) cloning refers to these cut-and-paste methods, which are still extensively employed today. There are several objectives of

molecular cloning, such as determining the expression pattern of a gene after a fish has been challenged by pathogens, and determining the putative involvement of specific lectins such as F-lectins in fish immunity.

17.2 Fish Lectins

Fish lectins are thought to facilitate pathogen identification, according to many studies. Membrane-associated lectins are vital recognition molecules that may aid in the establishment of beneficial mutualistic interactions with colonizing microbes or initiate innate and adaptive responses against potentially pathogenic microorganisms (Lino et al. 2013). Other activities of fish lectins include agglutination, immobilization with complement-mediated neutralization, and pathogen killing (Russell and Lumsden 2005) (Table 17.1).

17.3 Molecular Cloning of Fish Lectins

17.3.1 C-Type Lectins (CTLs)

CTLs are one of the most common lectins found in fish. CTLs are important for innate immunity in fishes. Their structures and functions have been thoroughly investigated in fishes and reported that they bind mono- and oligosaccharides with Ca^{2+}-dependent affinity. Pattern-recognition receptors (PRRs) called C-type lectins (CTLs) bind carbohydrate ligands in a Ca^{2+}-dependent way (Nurbaeva 2013). At least single carbohydrate recognition domain (CRD) with 115–130 amino acids form a characteristic double-loop structure in CTLs. This include collectins, proteoglycan core proteins, and selectins that offer various immunological functions in fishes. C-type lectins have been identified and characterized in some fishes, such as coho salmon *Oncorhynchus kisutch* (Yousif et al. 1995; Esmaeili et al. 2017),

Table 17.1 Lectin family in fishes

Fish species	Ligand	Lectin	References
Trichogaster trichopterz	*N*-acetyl glucosamine	C-type lectin	Fock et al. (2001)
Conger myriaster	β-Galactoside	Galectin	Fock et al. (2001)
Danio orerio	*N*-acetyl lactosamine	Galectin	Ahmed et al. (2004)
Aristichthys nobilis	Fucose	F-type lectin	Pan et al. (2010)
Silurus asotus	Mannose	Intelectin	Tsutsui et al. (2011)
Oplegnathus fasciatus	Rhamnose	Fish egg lectin	Kim et al. (2011)
Oreochromis niloticus	Mannose	C-type lectin	da Silva et al. (2012)
Ictalurus punctatus	Rhamnose	RBL	Thongda et al. (2014)
Larimichthys crocea	Galactose	C-type lectin	Ao et al. (2015)

orange-spotted grouper *Epinephelus coioides* (Ji et al. 2014), common carp *Cyprinus carpio* L. (Nakao et al. 2001), and rainbow trout *Oncorhynchus mykiss* (Zhang et al. 2000).

Regardless of how well proteins are able to bind sugars, CTLD refers to protein domains that are homologous to the prototypical C-type CRD or which have similar structure to that of the prototypic C-type lectin, prototypic CRD structure. For example, several CTLDs have developed the ability to selectively identify carbohydrates, inorganic ligands (Ca_2CO_3), and lipids (da Silva Lino et al. 2014). Various fish species, including the sea lamprey *Petromyzon marinus*, Japanese flounder *Paralichthys olivaceus*, and poisonous fish, have been shown to contain C-type lectins. *Thalassophryne nattereri* and *Ctenopharyngodon idellus* are two species of grass carp. Various fish species, including the sea lamprey *Petromyzon marinus*, the Japanese flounder *Paralichthys olivaceus*, and poisonous fish, have been shown to contain C-type lectins, *Thalassophryne nattereri* and *Ctenopharyngodon idellus* are two species of grass carp in which C-type lectin is found (Wang et al. 2017).

One of the research analyses came up with the molecular cloning of a C-type lectin in yellow catfish *Tachysurus fulvidraco*, which is the first reported cloning analysis with fish lectins. The entire sequence of CTL complementary (c)DNA was 685 nucleotides long. The open reading frame is thought to have encoded a 177-amino acid protein with an estimated molecular mass of c.y 20.204 kDa. A signal peptide and a single carbohydrate recognition domain with four cysteine residues and GlnProAsp (QPD) and TrpAsnAsp (WND) motifs were reported in that derived amino acid sequence. CTL was found to possess 56% of similarity to the anticipated lactose binding lectin from *Ictalurus punctatus*, a channel catfish. For cDNA cloning of *T. fulvidraco,* C-tType lectin in a previous study, a normalized complementary (c)DNA library of *T. fulvidraco* kidney was created (Ke et al. 2015). Using the National Center for Biotechnology Information's (NCBI) basic local alignment tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi), a partial sequence of a C-type-like lectin gene was discovered from the sequencing data. Using the Clontech (www.clontech.com) switching mechanism at 5′-end of RNA transcript (SMART) rapid amplification of cDNA ends (RACE) cDNA amplification kit, the putative 5′-end and 3′-end of the C-type-like lectin gene were obtained by 5′-RACE and 3′-RACE with gene-specific primers (for 5′-RACE: 5′-CAGCCAAAG CTGCTCCTG). The following conditions were employed for the PCR: 5 cycles of 94 °C for 30 s, 70 °C for 30 s, 72 °C for 3 min; followed by 25 cycles of 94 °C for 30 s, 68 °C for 30 s, 72 °C for 3 min. The PCR products were then inserted into the pMD18-T vector (TaKaRa; www.takara-bio.com) and sequenced. Then, sequence analysis was performed as reported. The C-type similar lectin's open reading frame (ORF) was identified using DNAStar'sEditSeq software (www.dnastar.com) (Cheng et al. 2013). The BLAST software was used to find nucleotide and amino acid sequences. SignalP 4.1 (Di Lella et al. 2011) and SMART (http://smart.embl-heidelberg.de/) were used to examine signal peptides and conserved domains. ClustalW 1.83 was used to accomplish multiple sequence alignments. The phylogenetic tree was built with 1000 bootstrap replications using the maximum likelihood technique included in MEGA 6. So, after cloning the purified recombinant CTL

(rCTL) from *E. coli,* BL21 was found to bind in a calcium-dependent way and agglutinate Gram-positive and Gram-negative bacteria. These findings demonstrated that CTL is a *T. fulvidraco* C-type lectin that function as a receptor for the innate immunity in fishes (PRR) with potent immunological functions (Tang et al. 2021).

Another report explained the molecular cloning of a C-type lectin in roughskin sculpin (*Trachidermus fasciatus*) that was discovered in the eastern coast of China, in Japan's Ariake Bay and the rivers that flow into it, and in Korean coastal waters. The immunological response of roughskin sculpins to pathogen exposure was investigated, and it was found that C-type lectins may play a primary role in roughskin sculpin immunity to a wide range of infections. For cloning, TfCTL1F1 and TfCTL1R1 gene-specific primer pairs were developed for TfCTL1 based on the expressed sequence tag (EST) acquired from random sequencing of a cDNA library of liver (Yu et al. 2013), and a specific PCR procedure was used: 94 °C for 3 min and 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min, and the centre fragments of TfCTL1 were produced. The PCR products were purified using gel electrophoresis and inserted into the pMD18-T vector (TaKaRa). After being transformed into competent *E. coli* cells, the positive clones were selected and sequenced in both directions by Sangon Company (Shanghai, China), and the resulting sequences were confirmed to be C-type lectin sequences using the online BLAST programme (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Arce et al. 2004). TfCTL1 full-length cDNA sequences were obtained using rapid amplification of cDNA ends (RACE). Fifty RACE were done using 50 primers linked with the gene-specific primer TfCTL1R1, whereas 30 RACE were performed with 30 anchor R paired with TfCTL1F1. The RACE reaction PCR methods were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. PCR products were cloned and sequenced as previously reported. ESTs and RACE were used to successfully clone a C-type lectin (TfCTL1). It shared significant structural features with other species' C-type lectins. TfCTL1 mRNA was found to be highly expressed in the liver. TfCTL1 expression was substantially upregulated after LPS exposure, showing that TfCTL1 was inducible. TfCTL1 recombinant exhibited bacterial and yeast agglutinating and binding capabilities. Finally, TfCTL1 was found to function as a pattern recognition receptor in innate immunity, where it is engaged in pattern recognition and pathogen elimination (Arce et al. 2004).

17.3.2 F-Type Lectin

One of the most recent families of lectins to be discovered and structurally described in fishes is the F-type lectin (FTL) family. A fucose recognition domain [F-type lectin domain (FTLD)] with a new jelly roll fold ('F-type') and specific carbohydrate- and calcium-binding sequence motif distinguishes members of the FTL family (Park et al. 2012). Immune surveillance and homeostasis have been linked to F-type lectins (FTLs). However, these and other lectin families are involved in a variety of

intracellular and extracellular activities such as glycoprotein folding, sorting, and secretion, cell–cell interactions, and signalling and transport in initial stages, tissue repair, and general cell function. While FTLs can have a single FTLD, which is often associated with one or more structurally and functionally different domains in a single polypeptide, they can also have several tandemly arrayed FTLDs. FTLs may be found in a wide range of organisms, from viruses to vertebrates (Vasta and Feng 2020). The F-type lectin fold is made up of a barrel with a jelly roll topology and two sheets of three and five antiparallel strands arranged opposite to each other. The connecting strands from the opposing sheets create five loops (CDRs 1–5) that surround the highly positively charged cleft that bonds the L-Fuc on the barrel's 'top' face. Two short antiparallel strands seal the barrel's 'bottom'. A substructure of three 310 helices securely coordinates a cation, most likely calcium, that helps to fold stability but, unlike C-type lectins (CTLs), does not directly interact with the carbohydrate. AAA subunits may create chloride-induced trimers with one cation (Ca^{2+}) per domain and many Cl on the threefold axis, and two trimers can form hexamers with opposing carbohydrate-binding surfaces.

Molecular cloning of F-type lectin from the rock bream, *Oplegnathus fasciatus,* was reported. The rock bream (*Oplegnathus fasciatus*) has two unique F-type lectins, RbFTL-1 and RbFTL-2, whose expression has been studied. Using EST analysis, two different F-type lectins (RbFTL-1 and RbFTL-2) were successfully cloned from the rock bream, *O. fasciatus* (Shao et al. 2018). RbFTL-1 and RbFTL-2 mRNA are mostly expressed in the liver and kidney, with lesser levels of expression in other organs (PBLs, spleen, gill, intestine, and muscle). These FTLs' expression was controlled in response to bacterial or viral challenges (Zarjou et al. 2019). RbFTL expression patterns differed after bacterial or RSIV activation. The expression profile of RbFTLs following infection shows that it is inducible and involved in the immune response of rock bream. RbFTL-1's full-length cDNA was 1204 bp long with a 945-bp open reading frame (ORF) encoding a 314 amino acid protein, whereas RbFTL-2's was 1614 bp long with a 951-bp ORF encoding a 316 amino acid protein. RbFTL-1 and RbFTL-2 mRNAs were found to be most abundant in the head–kidney and liver, respectively. RbFTL-1 mRNA transcript levels rose up to 5.0- and 2.8-fold in the head–kidney and trunk–kidney, respectively, compared to muscle, whereas RbFTL-2 mRNA transcript levels increased up to 12.0-fold in the liver. RbFTL-1 and RbFTL-2 expression were upregulated differently in rock bream treated with *Edwardsiella tarda*, *Streptococcus iniae,* and RSIV, with substantial increases at 1 and 3 h post-challenge compared to controls. RbFTLs are therefore inducible and susceptible to bacterial and viral infections. Increased RbFTL-1 and RbFTL-2 mRNA expression may assist to alert rock bream of harmful infections. Furthermore, both transcripts, particularly RbFTL-2, were significantly expressed in the liver 1 h after injection. In contrast, after 3 and 12 h after injection, the transcripts, notably the one encoding RbFTL-1, were highly expressed in the kidney. Multiple alignment of RbFTL-1 and RbFTL-2 FTL domains with other FTL domains revealed that their deduced amino acid sequences shared 37.6% and 20.1% identity with that of *A. japonica*; 16.8% and 11.4% identity with that of *D. rerio*; 55.8% and

52.6% identity with that of *D. labrax*; and 59.2% and 49.7% identity with that of *Lateolabrax japonicus*, respectively (Bae et al. 2012).

17.3.3 Galectin

Galectins are a group of lectins that bind to β-galactose and are homologous in the carbohydrate-binding site amino acid sequence. Galectins are part of the lectin superfamily as well (Dodd and Drickamer 2001), also known as β-galactose-binding proteins or S-type lectin-binding proteins. Galectins in vertebrates serve a variety of roles, including development, and innate and adaptive immunity. The amino acid sequence similarity between bbtGals and vertebrate galectins implies that amphioxus-galectins may have a function comparable to vertebrate homologs, which has been indirectly verified by the recombinant proteins' β-galactoside-binding ability (Yu et al. 2007). Many are divalent, and their ligands can form multimeric structures with them. Galectins, unlike C-type lectins, are not transmembrane proteins. Galectins accumulate in the cytoplasm and are typically released following cell damage; however, active immune cells and epithelial cells can also produce some galectins (Sato et al. 2009). Despite the fact that the number of genes differs between species, galectin structures are highly conserved in both invertebrates and vertebrates. They are involved in morphogenesis, cell proliferation, cell death, tumour functions, and a variety of other pathological activities. Since the first galectin was discovered in the electric organs of the electric eel (*Electrophorus electricus*), galectins have been discovered in a growing number of teleost fish species with a variety of biological roles (Kasai and Hirabayashi 1996). Molecular cloning of the Galectin-9 gene from *Labeorohita* was carried out in a study, where a galectin-9 (named LrGal-9) was characterized from *L. rohita*. Galectin-9 is a **β-galactoside**-binding tandem repeat galectin that regulates a variety of cellular activities, including cell adhesion and pathogen recognition (Mushtaq et al. 2018). Despite significant research into the role of human galectins in the immune system, little is known about fish galectins. A tandem-repeat galectin-9 from *Labeorohita* was discovered (and named LrGal-9), to examine the normal expression and immunological response of *Labeorohita* to infections. Its full-length cDNA was 1534 bp in length, encoding 291 amino acids (35.12 kDa), and shared the greatest 81% identity with *Danio rerio*'s galectin-9. The LrGal-9 found in this study lacked a signal peptide and a transmembrane domain, unlike other galectin-9 members found in fish (Matsumoto et al. 1998). Cloning of LrGalectin9 cDNA that is based on multiple alignments of *Sinocyclocheilus rhinocerous* (XM 016573382.1), *Danio rerio* (NM 200072.1), and *Cyprinus carpio* (XM 019116442.1) galectin-9 sequences degenerate primers for amplifying LrGal-9 cDNA were developed. The following PCR conditions were used to perform degenerate PCR amplification for a partial fragment of LrGal-9: an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and 1 min of extension at 72 °C, and a final elongation step of 72 °C for 5 min. Amplified PCR results were cloned and

sequenced into the pTZ57R/T vector (Thermo Scientific, Lithuania). Using the SMARTerTM RACE kit, the 5′ UTR (untranslated region) and 3′ UTR of LrGal-9 cDNA were obtained by rapid amplification of cDNA ends (RACE) (Clontech, USA). At M/s Bioserve Biotechnologies Pvt. Ltd. in India, the full-length cDNA of LrGal-9 was cloned into pTZ57R/T and sequenced. LrGal-9 expression profile revealed that it is abundantly expressed in the intestine and other tissues studied and that it is substantially upregulated in all the immunological tissues following *A. hydrophila* challenge. LrGal-9 (galectin-9) may serve as a pattern recognition receptor in *L. rohita*'s innate immunity, recognizing Gram-negative bacteria (Choi et al. 2021).

Other similar molecular cloning processes included a galectin-1 homolog in orange-spotted grouper, *Epinephelus coioides* where Galectin-1 occurs as a monomer or homodimer that is non-covalently bonded. Each type of shape has a distinct purpose. Galectin-1 dimerization is required for their role in mediating cell–cell or cell–ECM interactions. Galectin-1 is rich in thymus, kidney, skeletal muscle, smooth muscle, myocardial, sensory and motor neurons, and the placenta (Chen et al. 2016). A grouper (*Epinephelus coioides*) galectin-1 homolog (EcGel-1) was cloned, and its putative function in fish immunology was investigated. EcGel-1's full-length cDNA is 504 bp long, with a 408-bp open reading frame (ORF) encoding 135 amino acids and an atomic weight of 15.19 kDa. Cloning of *E. coioides* galectin-1 (EcGlec-1) full-length cDNA by RACE-PCR in this RNA was isolated from a frozen grouper liver sample using the SV Total RNA Isolation System (Promega) according to the manufacturer's instructions. Agarose electrophoresis was used to assess the quality of total RNA. The first-strand cDNA was synthesized from total hepatic RNA and used as templates for 30 RACE and 50 RACE using the SMARTTM RACE cDNA amplification kit (Clontech, USA). Primers were created based on the EST sequences found in the transcriptome libraries given by our lab. For the first round of PCR, primers Glec-1-50-224, Glec-1-30-64, and UPM (provided by the kit) were used. The first round PCR results were diluted 50 times and used as a template for the nested PCR, which was carried out using the particular primers Glec-1-50-304, Glec-1-30-198) and NUP (offered by the kit). The 30 and 50 RACE fragments were identified on 1% agarose gels, purified with the AxyPrep DNA gel extraction kit (AxyGEN), and sequenced. A novel S-type lectin (EcGlec-1) was successfully cloned from the grouper *E. coioides*, and its potential function in fish immunology was investigated. Muscle, heart, spleen, and head–kidney had the highest levels of EcGlec-1. With the addition of SGIV, LPS, and poly I:C, EcGlec-1 was upregulated. In the presence of 10 mM b-ME, rEcGlec-1 has the ability to hemagglutinate and attach to different bacteria. The findings might contribute to a better understanding of the EcGlec-1-host–pathogen relationship in fish innate immunity (Xu et al. 2008).

17.3.4 L-Rhamnose Lectin

L-Rhamnose-binding lectins (RBLs) have been isolated from a variety of fishes and invertebrates and have been shown to interact with a variety of bacteria, indicating that RBLs are involved in a number of inflammatory responses (Menaldo et al. 2017). The majority of RBLs are made up of two or three tandemly repeated characteristic carbohydrate-recognition domains (RBL CRDs), each of which has around 95 amino acid residues. Based on their domain design, hemagglutination activity for human erythrocytes, and sugar specificity against lactose, RBLs are divided into five categories (Hosono et al. 1999). Type I domains are three tandemly repeated domains. Type II is made up of two tandemly repeated domains plus an extra region. Types III and IV are made up of two tandemly repeated domains, although their hemagglutination activity and sugar selectivity vary. Type V has a single domain and homodimerizes through disulphide bond production. The effect of RBLs from chum salmon (*Oncorhynchus keta*), named CSL1, 2, and 3, on the peritoneal macrophage cell line from rainbow trout (*Oncorhynchus mykiss*) (RTM5) and an established fibroblast-like cell line derived from gonadal tissue of rainbow trout (RTG-2). CSLs were bound to the surface of RTM5 and RTG-2 cells and induced proinflammatory cytokines, including IL-1β 1, IL-1β 2, TNF-α1, TNF-α2, and IL-8 in both cells by recognizing globotriaosylceramide (Gb3) (vanSetten et al. 1996). In addition, CSLs had an opsonic effect on RTM5 cells, and this effect was significantly inhibited by L-rhamnose, indicating that CSLs enhanced their phagocytosis by binding to Gb3 on cell surfaces. It was the first finding that Gb3 plays a role in innate immunity by cooperating with natural ligands, RBLs (Watanabe et al. 2009).

Another rhamnose-binding lectin from snakehead *Channa argus* was cloned utilizing rhamnose-binding lectin (RBL) gene and its promoter region. The snakehead rhamnose-binding lectin (SHL) gene spans 2382 bp from the transcription start site to the end of the 30 untranslated region (UTR) and comprises nine exons and eight introns. The SHL transcript's open reading frame (ORF) is 675 bp long and encodes 224 amino acids. SHL's molecular structure is made up of 2 tandem repeat carbohydrate recognition domains (CRD) with 35% internal similarity with the use of molecular cloning. SHL (snakehead rhamnose-binding lectin) mRNA transcripts were found in the head–kidney, posterior–kidney, spleen, liver, gut, and heart, along with muscle and the ovary (Jia et al. 2010). White-spotted charr WCLs, steelhead trout STLs, and ponyfish PFLs have tissue-specific expressive motifs. Different forms of RBLs should exist in particular species of fish that have developed and adapted to their surroundings, according to the expression pattern of the snakehead SHL gene. The Cloning SHL cDNA sequences by RACE-PCR full-length cDNA was generated following the manufacturer's instructions using the BD SMARTTM RACE (Rapid Amplification of cDNA Ends) cDNA Amplification Kit (Jia et al. 2010). The 50 UPM and SHL-R1 primers were used in the main PCR to extract the 50 segments of SHL cDNA. The thermal cycle was as follows: 94 °C for 2 min, followed by 10 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 45 s,

with the annealing temperature reduced by 1 °C for each cycle, and 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by 72 °C for 6 min. The secondary PCR was performed using 50 nested primers and SHL-R2, as well as 1 μL of the original PCR mix, using the cycling protocol: 94 °C for 2 min, then 30 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 45 s, followed by 72 °C for 6 min. To create a first-strand cDNA, a total RNA sample from 50 RACE was utilized. Adapter-dT17 primer was used to reverse transcribe PolyA+ RNA to cDNA. Except for a 1-min extension at 72 °C, the PCR conditions and procedures were the same as reported in 50 RACE using the 30 adaptor primer and SHL-F1/F2. Cloning SHL genomic sequence and promoter region using phenol-chloroform technique was used to purify genomic DNA from snakehead muscle. The area of the SHL gene, including introns, was amplified using primer pair SHL-GF/GR based on the SHL cDNA sequence. The BD AdvantageTM 2 PCR Kit was used to complete the PCR experiments (Clontech). The cycling regimen was 94 °C for 2 min, 32 cycles of 94 °C for 30 s, and 3 min at 68 °C, followed by 10 min at 68 °C. The 50 flanking area was acquired by building genomic libraries using the Universal Genome WalkerTM Kit utilizing a genome walking method (Clontech). From the 50 ends of SHL cDNA, SHL-P1/P2 primers were constructed, priming upstream amplification by two rounds of PCR (Jia et al. 2010). The main PCR was performed as; 94 °C for 2 min, followed by 6 cycles of 94 °C for 30 s, 72 °C for 3 min with the annealing temperature reduced by 1 °C for each cycle, and 30 cycles of 94 °C for 30 s and 67 °C for 3 min, followed by 67 °C for 10 min. About 1 μL of the original PCR mix was used for the secondary PCR, which consisted of 20 cycles of 94 °C for 30 s and 67 °C for 3 min, followed by 67 °C for 10 min. The findings suggest that SHL expression may be increased in response to inflammatory stimuli such as lipopolysaccharide (LPS), interleukin 6 (IL-6), and interferon gamma (IFN-c). The SHL mRNA transcript was found in the head–kidney, posterior–kidney, spleen, liver, gut, heart, muscle, and ovary. There is no tissue-specific expressive pattern that differs from previously described STLs, WCLs, and PFLs, implying that distinct forms of RBLs exist in species-specific fish that have developed and adapted to their surroundings. The cloning aids in the identification of fish-RBL family members and also contributes to the understanding of the RBL family's evolution (Jia et al. 2010) (Fig. 17.1).

17.3.5 Pentraxins

Pentraxins are made up of numerous subunits that range in size from 20 to 25 kDa and have one CRD per subunit and class of conserved PRMs that have a radial pentameric structure (Parente 2019). Short and long pentraxins are distinguished: C-reactive protein (CRP) and serum amyloid-P (SAP) are prototype short pentraxins, whereas pentraxin 3 (PTX3) is the prototypical long pentraxin. CRP has the ability to opsonize apoptotic cells and facilitate their clearance. CRP binding to phosphocholine was studied, and the amino acid residues Lys57, Arg58, and

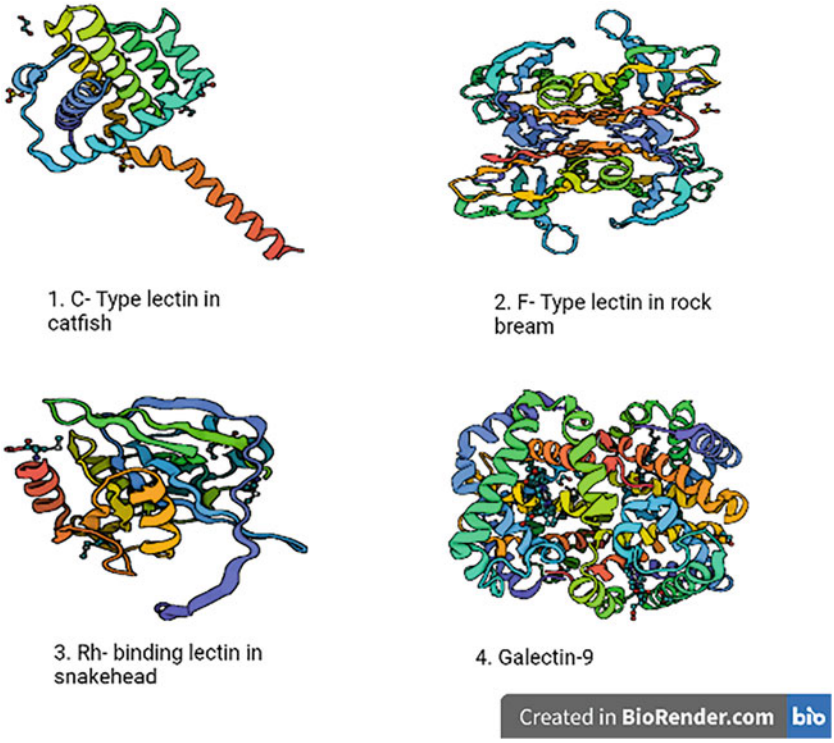

Fig. 17.1 Crystal structure of (1) C-type lectin (5W63) 2.436 Å; (2) F-type lectin (1SN2) 1.75 Å; (3) Rh-binding lectin (2GEF) 2.2 Å; (4) Galectin (2R1H) 1.9 Å, developed through X-ray diffraction

Trp67 were discovered to be important for the interaction (Agrawal et al. 2020). In addition to binding phosphocholine, it has lectin-like characteristics such as Ca^{2+}-dependent binding to galactans and similar structures. Hepatocytes generate CRP, a 25-kDa acute-phase protein, in response to interleukin (IL)-6. It was discovered because of its calcium-dependent capacity to bind the C-polysaccharide of *Streptococcus pneumoniae* (Jarva et al. 2002). In the presence of phosphorylcholine which is found on apoptotic cells, is also a component of the capsule of certain bacteria. CRP activates complement C1q. Pentraxins demonstrated opsonin activity in snapper *Pagrus auratus*, indicating a functional role in the host defence fish (Reite 2005). They were also detected in *pangasius Pangasianodon* hypophthalmus serum (Huong Giang et al. 2010) and Atlantic cod, *Gadus morhua* serum. Pentraxins have been isolated from teleosts such as channel catfish, carp, snapper (*Pagrus auratus*), Atlantic salmon, common wolffish (*Anarhichas lupus*), cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*), and rainbow trout.

Table 17.2 Molecular cloning in fish lectin and their applications

Lectins	Species	Genes	Applications	References
Galectin-1	*Epinephelus coioides*	EcGle-1	Host–pathogen relationship in fish innate immunity	Lam et al. (2011)
C-lectin	*Tachysurus fulvidraco, Trachidermus fasciatus*	CTL/ TfCTL1	Innate immunity, pathogenic activity, cell–cell interactions	Yang et al. (2013)
F-lectin	*Oplegnathus fasciatus*	RbFTL	Innate immunity	Cho et al. (2014)
Rhamnose-binding lectin	*Channa argus*	SHL	Identification of fish RBL family and their evolution	Zhou et al. (2020)
Galectin-9	*Labeo rohita*	LrGal-9	Serve as a pattern recognition receptor	Yu et al. (2021)

17.3.6 Calnexin

Calnexin is a transmembrane protein that interacts with nascent glycoproteins' mono-glycosylated, trimmed intermediates of N-linked core glycans. Calnexin is a protein that interacts with glycans in membrane proximal domains (Zimmer et al. 2001) and has recently been discovered in channel catfish. Calnexin (IP90/P88) is an endoplasmic reticulum membrane protein that binds newly produced N-linked glycoproteins in the ER during their folding, including the MHC class I molecule. It was discovered to interact with partially folded MHC class I molecules, T-cell receptors, and membrane immunoglobulins (Natarajan et al. 2002). Soluble counterpart calreticulin, participates in the calnexin/calreticulin cycle, which is responsible for folding and quality control of newly produced glycoproteins before their export from the ER. Calnexin has also been linked to ER calcium regulation, phagocytosis, and cell apoptosis resistance (Bedard et al. 2005). Calnexin crystallization showed two different luminal domains: a globular domain containing the lectin binding site and an extended arm domain including two tandem proline-rich motifs that bind to the ER. The C-terminal domain is extremely acidic and contains a protein kinase-dependent phosphorylation site that may play a role in chaperone function regulation. Calnexin's ability to stabilize both MHC class I and MHC class II molecules is a well-studied function (Paulsson and Wang 2003) (Table 17.2).

17.4 CRISPR Techniques in Fish Lectin Research

The CRISPR/Cas9 system is a strong, effective tool for editing genomic DNA sequences, including gene knockout in fish, using RNA-guided site-specific DNA cleavage. The system is made up of a guide RNA (gRNA) that specifies the targeted sequence in the genome and the Cas9 DNA endonuclease enzyme (Chang et al. 2013). The CRISPR/Cas9 system is engineered to target any sequence in the genome

(Patmanathan et al. 2018) with multiple characteristics such as (1) cheaper cost, (2) simpler engineering, (3) more precise binding of guide RNA to target sequence and fewer off-target mutations, (4) multiple sequences can be targeted with various gRNA at the same time, (5) high mutagenesis rate in genes, and (6) enhanced germline transmission rate of mutations up to sixfold. Gene knockout has been used to study these gene functions in vivo. The CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) is a potent tool for editing genomic DNA sequences to change gene function. Due to interactions of gene products in multiple metabolic pathways resulting in pleiotropic effects or epistasis, knocking out or silencing a gene may impact not only the primary phenotype, but also one or more secondary phenotypes. Guide RNA (gRNA)/Cas9 protein microinjection into catfish embryos may alter hatching, embryo growth, and early fry survival (Pramanik et al. 2021). The increased mortality of implanted embryos might be attributable to the microinjection technique, off target mutations caused by the gRNA/Cas9 protein, or pleiotropic effects of target gene deletion. Since the CRISPR/Cas9 technology has opened the door to precise genome editing, it is critical to evaluate the impact of gRNA and Cas9 on the survival and hatchability of microinjected catfish embryos in order to develop more efficient gene editing studies. The number of embryos and amount of gRNA/Cas9 protein to be injected to create enough founder fish for functional genomics research with minimal off-target mutations may be accurately estimated by studying such effects (Hongbao et al. 2016).

Guide RNAs/Cas9 targeting the toll/interleukin 1 receptor domain containing adapter molecule (TICAM 1) gene and the rhamnose-binding lectin (RBL) gene were microinjected into the yolk of one-cell embryos after the injection volume was adjusted. Indels were confirmed by DNA sequencing, indicating that the gene knockdown was effective (Elaswad et al. 2018). Frameshift and shortened protein owing to premature stop codons were among the anticipated protein sequence changes caused by these mutations. Regular microinjection of catfish embryos is technically challenging and time consuming; however, for channel catfish embryos, a fast and effective CRISPR/Cas9 protein microinjection procedure is provided. Because the CRISPR/Cas9 protein solution is injected into the yolk of one-cell embryos, this procedure required less time and skill. In 1 h (about the time it takes for the initial cell division to begin), hundreds of fertilized eggs can be injected. Two disease susceptibility-related genes, the toll/interleukin 1 receptor domain-containing adapter molecule (TICAM1) gene and the rhamnose-binding lectin (RBL) gene, were knocked out in channel catfish to verify the technique. TICAM-1 is a component of the toll-like receptor (TLR) 3 signalling pathway. The mutation rate of both genes, as well as the mortality rate of channel catfish embryos, increased when the concentration of TICAM-1 gRNA/Cas9 protein was increased. When different quantities of gRNA/Cas9 mRNA and gRNA/Cas9 protein were injected into zebrafish, a similar finding was reached. The researchers determined that increasing the amount of Cas9 mRNA injected into zebrafish embryos resulted in an increase in hazardous phenotypes ranging from mortality within a few hours to heart, nervous system, and axis development abnormalities. The increased mortality

in the two trials might be due to off-target effects, direct toxicity from higher RNA and protein quantities, or both, and the specific cause cannot be determined without more research. The prevalence of distinct types of mutations reveals when CRISPR/Cas9 protein-induced mutations occurred throughout development. The fact that there were fewer types of mutations with a high frequency suggested that Cas9 protein cleaved DNA targets early in development. Low-dose RBL embryos showed four different mutations. The two forms of mutation seen at medium and high doses point to a bi-allelic mutation of the RBL gene, in which a DSB in one of the two alleles caused a distinct type of mutation. When the tyrosinase gene was targeted with CRISPR/Cas9, bi-allelic alterations were seen in zebrafish (Yen et al. 2014). The pigmentation of tyrosinase mutant embryos was mosaic, indicating that some cells with the wild-type tyrosinase gene generated pigmentation. The three gRNA doses produced statistically equivalent embryo survival in RBL, which differed from the iCTRL therapy. The hatching fraction in RBL was significantly reduced by the microinjection procedure, whereas in TICAM 1, the hatch percent in the nCTRL group was still 22% higher than the iCTRL group, but the difference was not significant due to the large variation in hatch percent among the replicates. When compared to the iCTRL group, injection of gRNA/Cas9 protein reduced significantly the hatch percent in RBL, and increasing the dosage from low to moderate significantly reduced the hatch percent, which is primarily affected by embryo fatalities in each treatment. Although it was not significant for TICAM 1, the hatch percent was reduced by more than 30% when the dosage continued to increase from low to medium. When compared to the nCTRL, fry in most treatments had significantly shorter mean survival times, indicating that microinjection and/or gRNA/Cas9 dosage may have an effect on early fry survival. In TICAM 1, microinjection procedures significantly reduced early fry survival, but gRNA/Cas9 protein had no effect. The gRNA/Cas9 protein reduced fry survival in RBL. Following a bacterial challenge with *Edwardsiella ictaluri*, TICAM-1 was substantially increased in channel catfish, while it was downregulated in blue catfish, a species resistant to *E. ictaluri*. RBL has a significant impact on early infection with *Flavobacterium columnare*, the causative agent of columnaris illness, and was associated with rapid and strong increase in a columnaris-prone channel catfish strain against a columnaris-resistant strain (Beck et al. 2012). Finally, the creation of a reliable and effective procedure for targeted gene editing in channel catfish is critical for functional genomics research, especially now that the genomic resources have been expanded by the recent sequencing of the channel catfish genome. This study found that increasing the dosage of gRNA/Cas9 protein can increase the mutation rate. Higher dosages resulted in homozygous and heterozygous biallelic mutations, but no wild-type alleles were found, implying gene knockout in both chromosomes at the one-cell stage. Higher doses, on the other hand, decreased embryo survival and hatching. Because of the low dosage, both mutated and wild-type alleles were found in the embryos. Future study is needed to establish the rate of mutation transmission in the germline, as well as the impact of TICAM-1 and RBL gene deletion on channel catfish immune response and disease resistance. The techniques outlined here are straightforward, quick, and effective in achieving

gene knockout. The efficacy of CRISPR/Cas9 knockdown via microinjection was demonstrated utilizing two genes: TICAM-1 and RBL. The procedure can be changed to incorporate different biologically active chemicals depending on what is being injected. It may also be tweaked to microinject eggs from other catfish species or fish with comparable egg shape and content (Lange et al. 2017).

Hence, the CRISPR/Cas9 system is a potent tool for editing genomic DNA sequences, including specific gene knockout in fish, using RNA-guided site-specific DNA cleavage. This efficacy of CRISPR/Cas9 knockdown via microinjection was demonstrated utilizing diverse genes across fish species that helps in future analysis and discoveries. The most significant advantages of CRISPR in comparison to other gene-editing technologies are probably its simplicity and effectiveness, which is primarily due to its RNA-based nature. CRISPR is significantly less labour-demanding and less expensive, thereby utilizing the extended CRISPR toolbox to mediate additional genome mutations, including gene expression regulation with CRISPRi and CRISPRa. It mediates the insertion of single-nucleotide variations with base editing, or even prime editing. Furthermore, for the vast majority of applications, the CRISPR system offers the ideal mix of accessibility, efficiency, and accuracy for gene editing, and it has the added benefit of being able to target numerous loci at the same time—or to do high-throughput, genome-wide functional tests.

17.4.1 Limitations in the Use of CRISPR/Cas

Despite the benefits of CRISPR/Cas, realizing the technique's full potential in fish farming is restricted by several technical obstacles, which are outlined from both the genetic and application viewpoints.

Genetic perspective: Trait-related genes must be clarified because the genetic dissection of aquatic creatures lags behind that of humans and plants, wherein trait-related genes must be identified. To put it another way, which gene should be targeted? The process of identifying target genes, whether by quantitative trait locus (QTL) mapping or marker assistance, is typically time-consuming. Although resequencing technology has made the procedure easier, the discovery and validation of polygenic determined traits still make accurate identification of gene loci difficult.

Among aquatic creatures, fish are the most abundant species. With the salmon-specific fourth round, this problem is extended (Ss4R). The method in which duplication impedes the editing efficacy of CRISPR (gene editing) techniques; for example, finfish has been explored, and a comparison of genes with different copies in the genome may be analysed to explain this issue in detail (Glasauer and Neuhauss 2014).

Application perspective: The egg membrane reduces the success rate of microinjection in oviparous fish and ovoviviparous fishes; and there is currently no approved gene editing platform. There are no standard procedures as the aquatic creatures have unique characteristics that necessitate species-specific design, such as needle type, injection dose, and so on. Due to a limitation in the amount of known cell lines and the tiny size of the egg and embryo in crustacea and molluscs, only *Crepidula fornicate, Exopalaemon carinicauda*, and *Crassostrea gigas* have had successful genome editing. Many aquatic species have very long generation intervals, making the acquisition of mutant homozygous individuals throughout the GE process time intensive (Perry and Henry 2015).

Off-target impact identification in model organisms focuses on knockout efficiency in order to enhance CRISPR/Cas design. Off-target effects in aquatic creatures should be studied as a food resource, as well as the influence of adding additional genes via transgenesis or cisgenesis. This necessitates a more thorough evaluation, taking into account both off targets in the genome and possible risks connected to food quality or safety (Blix et al. 2021).

17.5 Conclusion

Fish lectins have a wide range of structural and functional properties, including diverse carbohydrate specificity that are unique and vast in different species. The discovery, isolation, and physicochemical characterization of various fish lectins are expanding at an exponential rate. Identification of molecular characteristics, particularly fish lectins with downstream effects, and, more critically, confirmation of their importance in clinically relevant fish illness, remain largely unachieved. The presence of such lectins play an important role in innate immunity, especially in the activation of the complement pathway. Molecular cloning and CRISPR in fish lectins have several advantages like contributing a better understanding of several gene interactions like EcGlec-1-host–pathogen relationship in fish innate immunity; molecular cloning in the identification of certain fish genes under the light of evolution; determining the expression pattern of a gene after a fish has been challenged by pathogens; determining the putative involvement of novel F-lectins in fish immunity; recognizing Gram-negative and Gram-positive bacteria, mediating bacterial and yeast agglutination; and binding capabilities engaged in pattern recognition and pathogen elimination. Notably, the functional impairment of specific lectins caused by gene mutations is most likely the cause of species susceptibility or resistance to many infectious fish diseases which should be dealt in detail. However, little is known about the molecular cloning and gene editing methods of significant lectins that uplift aquatic immunity in comparison to their expression. For example, Pentraxins and Calnexin are significant fish lectins in which molecular cloning and gene editing techniques have not been reported in detail. High aquaculture production with disease resistance is in great demand for the commercial

exploitation. And so, in-depth analysis of such genome editing techniques utilizing current genomic and cloning methods on fish lectins will provide better understanding of the functions in their recognition and role as effector molecules assisting both innate and adaptive immunity in fishes.

Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 18
Future Perspective of Fish Lectin Research

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Abstract Lectins are proteins which have the ability to bind with specific carbohydrates and have the capability for preventing or curing various infectious diseases in many different forms such as biomedical tools in drug designing and also as biopharmaceuticals. This gives rising concern regarding epidemics and drug resistance, eventually this opens the way for the future perspectives on the antimicrobial (antibacterial and antiviral) applications of lectins. The recent findings show that lectins can be used as a biological tool in the field nanosensors which are then used for the drug delivery systems, antibiotic susceptibility tests for the treatment of drug resistant bacteria and diagnosing infectious viral diseases and for producing vaccine adjuvant. Even though the development of new remedies or medicines is not cost effective when compared to the production of lectins, it concludes that it is worth investing on the lectins with antimicrobial characteristics than on the antibiotics, and this paves a path for the emergence of epidemics. As an alternative to parenteral administration of biology, lesser risk factor routes must be developed for these purposes. For example, the development of anti-glycan neutralizing antibodies is difficult because of the carbohydrates low immunogenicity, so as a result lectins can be produced with no trouble, and it also has a broad spectrum activity. So, this chapter discusses about the future perspectives of fish lectin research in various fields such as drug designing, immunology, pathology (both human and animal) and several other applications. The integration of omics technologies, especially proteomics technology, might take these applications to a whole new level and more successful in the near future

Keywords Fish lectins · Antibiotics · Genomics · Proteomics · Antimicrobial activity · Anticancer activity

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Abbreviations

BCA	Boodlea coacta lectin
BGL	Boletopsis grisea lectin
COVID-19	Coronavirus disease 2019
CSL	Cerebral soluble lectin
CTL	C-type lectins
CV-N	Cyanovirin-N
DDS	Drug delivery system
DNA	Deoxyribonucleic acid
FTL	F-type lectins
Gb3	Globotriose
HIV	Human immunodeficiency virus
MBL	Mannose-binding lectin
MM46	Mouse mammary cells
PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
RBL	Rhamnose-binding lectin
RNA	Ribonuleic acid
SARS	Severe acute respiratory syndrome

18.1 Introduction

Lectins are also called agglutinins or haemagglutinins; these are ubiquitous proteins which do not have any immunological origin and catalytic activity. When it comes to distribution, the lectins are widely distributed in both prokaryotic and eukaryotic organisms; this includes algae, fungi, crustaceans, vertebrates and invertebrates, and the lectins obtained from these sources have the ability to develop immune responses against the infectious pathogens as a defence mechanism (Peumans and Van Damme 1995). Through complementary non-covalent interactions, lectins have the ability to accommodate the monosaccharides, glycoconjugates and oligosaccharides by carbohydrate recognition domain. Without manipulating the covalent structure of any previously recognized glycosyl ligands, lectins have the potential to reversibly bind to carbohydrates molecules (André et al. 2015). There are several types of lectins from the marine resources, which are classified based on its structure, function and physicochemical characters. Some of the well-known types are galectins (S-type), C-, L-, M-, P-, I-, F-, R-type lectins, etc. (De Hoff et al. 2009). As per the pattern recognition receptors (PRRs), the C-type lectins have the ability to identify glycans from various pathogens like parasites, bacteria, fungi and viruses as pathogen-associated molecular patterns; therefore, this C-type lectin develops innate and adaptive immunity in many organisms (Mayer et al. 2017). Interaction of lectins with the cell wall components of invasive pathogens is responsible for the antifungal

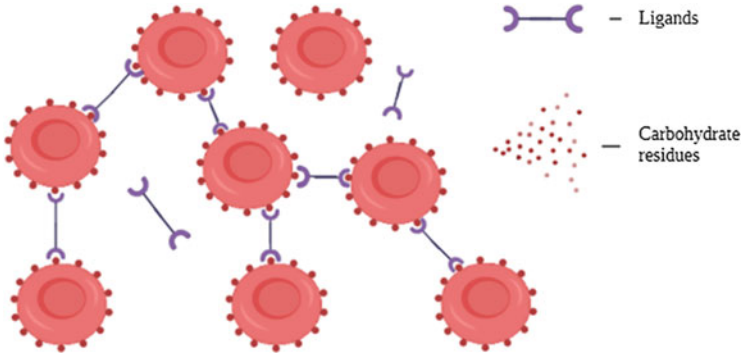

Fig. 18.1 Lectin detection through haemagglutination activity

and antibacterial activities. By forming a pore, the second functional domain has the ability to alter the cell membrane permeability or by cell wall dissociation as per de novo synthesis (Breitenbach Barroso Coelho et al. 2018). Due to the participation of lectins in host cell–virus fusion mechanism, it acts as an antiviral agent by resisting the entry of viral particles (Mitchell et al. 2017). Several viral envelopes are composed of high-mannose glycan structures such as in human immunodeficiency virus (HIV); consisting of 120 envelope protein, through conformational changes leads to fusion component state of the viral particle (Koharudin and Gronenborn 2014) (Fig. 18.1).

In several viral infections like hepatitis C, corona virus, influenza A and B and herpes simplex 1 and 2, the lectins impose neutralization activities, so the lectins act as a potential therapeutic agent (Mazalovska and Kouokam 2018). In the current scenario like coronavirus disease 19 (COVID-19) pandemic, a search for antiviral agents is emerging in order to cure COVID-19. As we talk about the COVID-19, mannose-binding lectins are one of the potential inhibitors of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which blocks the viral entry because protein S contains oligomannose in its binding sites (Watanabe et al. 2020). The development of new medicines by using lectins can open door for biopharmaceuticals and drugs with therapeutic mechanisms or even have them as molecular targets (Ribeiro & Mahal 2013; Roy et al. 2016). Here in this chapter, we discuss about the potential of lectins as antiviral, antifungal and antibacterial agents and its applications to indicate the current scenario as well as the future aspects of using lectins in various fields, especially in drug designing and discovery. Patents are important documents to address the state-of-the-art literature of a topic, and this lack of information on antimicrobial lectins is particularly important nowadays, given the rising threat of drug resistance and epidemics.

18.2 Distribution of Fish Lectins

Fish lectins are commonly found in the fish's serum, gills, surface mucous, egg surface, haemolymph and other organs. It is well known that the skins of various aquatic animals, particularly fish, have prosperous source of lectins, but many of these lectins are still unidentified and unreported. In the near future, these unidentified and unreported lectins can be identified by implementing omics technologies. The fish lectin was firstly reported and discovered from the exterior mucus of fish (*Genypterus blacodes*), belonging to the family *Ophidiidae* (Oda et al. 1984). Lectins have been isolated in large quantities from the entire digestive system as well as from several other sources (Fig. 18.2).

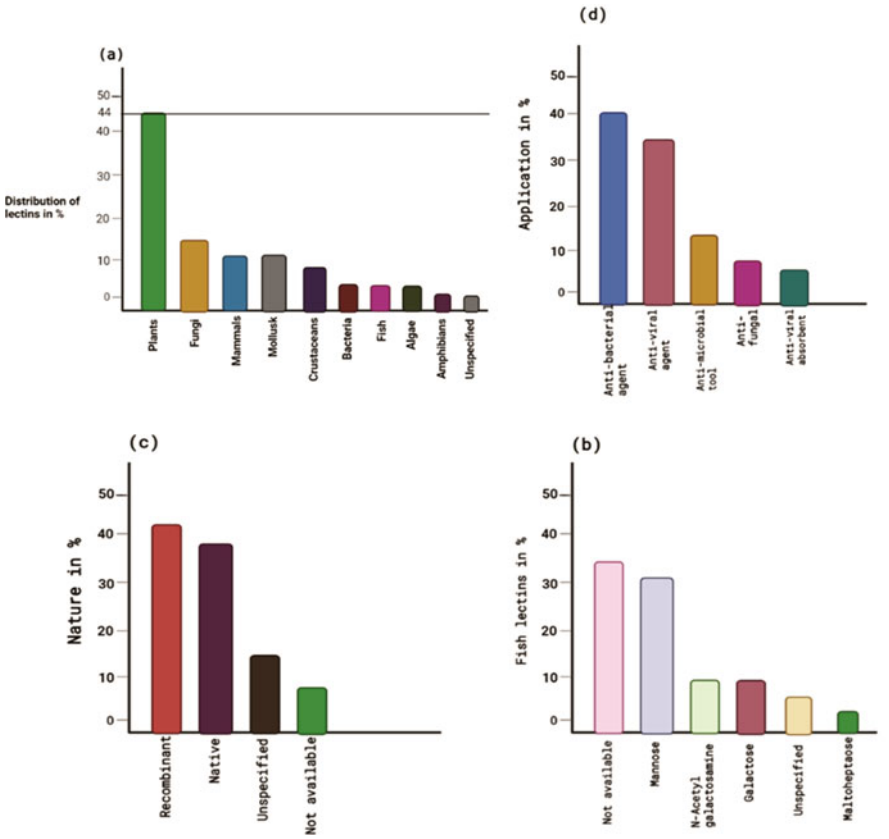

Fig. 18.2 Distribution of lectins. (**a**) Distribution of lectins, (**b**) fish lectin specificity, (**c**) nature of fish lectins, (**d**) application of fish lectins

18.3 Future Perspective of Fish Lectin Research

There is a big scope for researches in the field of fish lectin, because only limited number of lectins are isolated and analysed, but many of them remain unidentified, and such unreported lectins can be discovered and used for various purposes in the near future (Fig. 18.3).

In the fish lectin research, the applications and nature of lectins are left unexplored for all these years, but through recent studies, the role of lectins in research is getting greater attention. Lectins are categorized under the branch of study called lectinology, which is a recently emerging study to understand the role, application and function of lectins in various fields, but there are only limited concepts in it. Lectins of animals, plants and other species are isolated and exploited. It shows that lectin has various perishable uses to the society. Likewise, many lectins are present in fishes, and some of them have been analysed, and many left unutilized. Recent discoveries reveal that fish lectins have enormous advantages. These lectins are cost effective for isolation and production when compared to the production cost of antibiotics against specific diseases, and also the results are almost reliable to that of antibiotics. Also these lectins are isolated from the natural sources rather than antibiotics, which as a result do not have side effect to the host organism.

The anomalous nature of lectins makes it to react and agglutinate with saccharide group. Specific lectin reacts only with specific carbohydrates to be used for qualitative and quantitative assays on saccharides. Lectins can be used to separate a

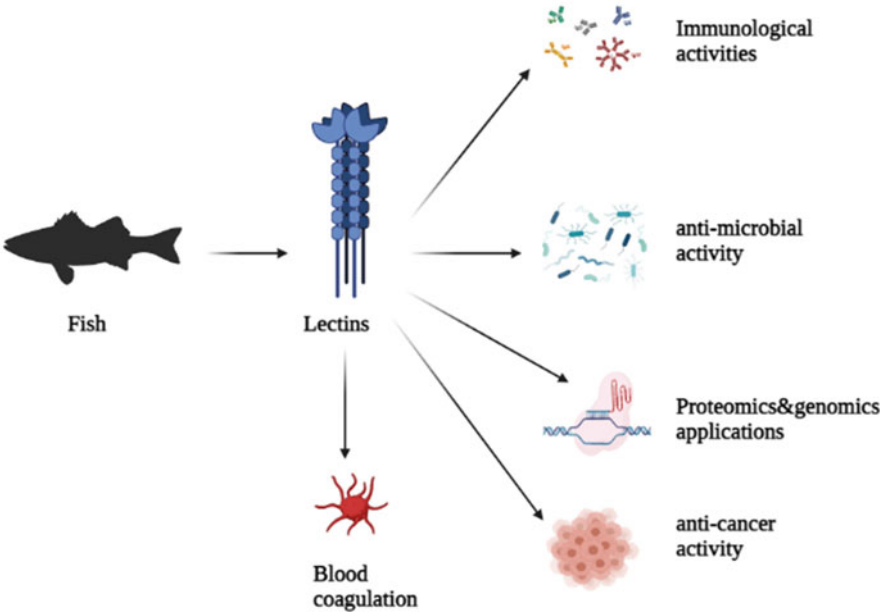

Fig. 18.3 Future fields of fish lectin research

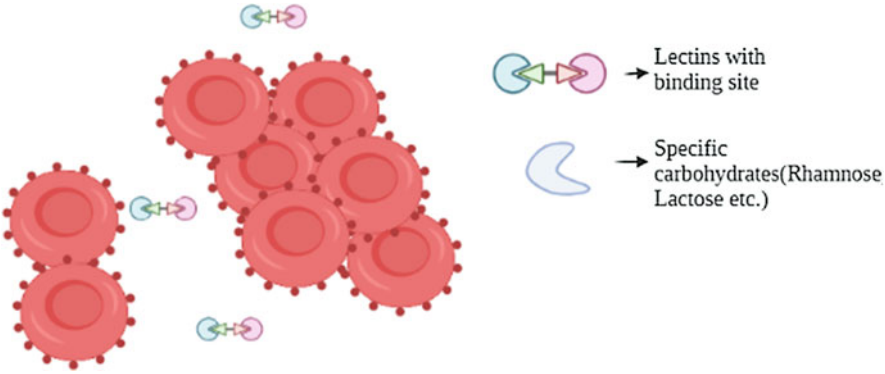

Fig. 18.4 Inhibition of haemagglutination to assure lectin presence and its specificity with carbohydrates

carbohydrate from a complex mixture and also can be used to nullify the interference of carbohydrates in an experiment. In molecular biology, it can be used as an isolation and analysation tool. In forensic science, there are various issues in finding out the lawbreakers through forensic samples like hair, nail and blood. As fish lectins have the agglutination property, they can be used to identify the victim's blood group and also have the potential to isolate specific blood type from contaminated blood. The advantages of lectins have been full-fledged from the lab to the industry level. For instance, Hjch is a CTL (C-type lectin) isolated from the epidermal tissues of Japanese bullhead shark *Heteroclontus japonicas*. It is more like a venomous substance, and with proper understanding, these lectins can be used as an anti-venom agent. Fish lectins are used in the separation of carbohydrate from the wasted and spent off medium with higher carbohydrate content can be done with its binding and agglutinating capability, and as a result, specific saccharides were isolated and purified (Ao et al. 2007) (Fig. 18.4).

18.4 Applications of Fish Lectins

18.4.1 Lectins as Antimicrobial Agents

The recent studies show that the marine lectins have the ability to bind and agglutinate with several organisms such as algae, fungi, viruses, planktons, especially diatoms and dinoflagellates and also bacteria of different species. Therefore, these lectins act in analogous manner to prevent and eliminate the invading pathogens. A C-type lectin obtained from the amphioxus has the potential to kill the microbial strains *Staphylococcus aureus* and *Saccharomyces cerevisiae* by binding to the glucan and peptidoglycan, respectively, which are basically the cell wall polysaccharides. Rhamnose-binding lectins (RBLs) obtained from the *Oncorhynchus mykiss*

fish eggs interact and agglutinate with Gram-positive and Gram-negative bacteria by identifying the cell surface and PAMPs (Tateno et al. 2002). The RBLs have the potential to recognize the O-antigen, which is basically the immunodominant structure when exposed to external conditions; it has high variability on different bacterial strains, and the mechanism behind is the ability for the diverse carbohydrate recognition. Additionally, these RBLs have the tendency to bind with microsporidian fish pathogens. The RBL receptor expression can be found after inflammatory stimulation on the peritoneal macrophages of fish. Recently in rainbow trout macrophage, the cerebral soluble lectins (CSLs) have the capability to produce radical oxygen species (Watanabe et al. 2008). The lectins that are obtained from the coho salmon shows antimicrobial activity against the bacterium *Aeromonas salmonicida* which is the causative pathogen for tuberculosis. The activity of several lectins against specific microbes not only terminates or kills the microbes but also develops an innate immunity in the host organism rather than adaptive immunity. So in the future, these lectins can be used to develop an immunomodulatory or immunostimulatory molecule, which enhances the innate immunity in humans and certain other animals.

The lectins that are isolated from the sources like fishes not only show antimicrobial activity in fish but also show effective activity against the human pathogens. Lectins of some fishes produce immunity against selective pathogens. F-type lectins (FTLs) and GANL isolated from the gills of bighead carp (*Aristichthys nobilis*) have high potential in resisting against *Vibrio harveyi.* For example, ACTL and BGL from gourami (*Trichogaster trichopterus*) improve the innate immunity against *Aeromonas hydrophila*, which is a common fish pathogen. Some of the fish microbes are pathogenic to humans, and thereby, it cause zoonotic disease in humans. Lectins of certain type obtained from several fish could help to prevent and treat these zoonotic diseases.

As we discussed before, the first ever discovered lectin is from skin mucous. Lectins in fish mucous membrane play a crucial role against these infectious agents. Therefore, the mucous lining acts as a primary defence against most of the microbes in fishes. Fishes are exposed to various types of antigenic and inflammatory stimulus in the aquatic system. Lectins isolated from the fish mucous shows chemotaxis activity towards various bacteria. For instance, most of the serotypes of *Vibrio anguillarum* are chemotactic towards skin mucous of rainbow trout, Atlantic cod, etc. Especially Catfish mucus exhibits chemotactic activity against *Flavobacterium columnare*. Analysing the functional activity of these lectins paves the way to bio-medicinal applications. This concept will be also served as a tool in microbiology researches and immunological researches.

18.4.2 Lectins as Anticancer Agents

Lectins are used in the cancer cell recognition, mitogenic cytotoxicity, cell adhesion, apoptosis and signal transduction throughout the membrane as surface markers. In

both in vitro and in vivo methods, these lectins have the potential to modulate the proliferation, growth and apoptosis of malignant and pre-malignant cells. These studies prove that the lectins can be used in diagnosis, treatment and prevention of chronic diseases like cancer (Ghazarian et al. 2011). The lectins obtained from *Megabalanus rosa* (BRAs) shows antitumour activity on in vivo method (Yamazaki et al. 1983). After the in vitro incubation with the BRAs, the mouse mammary tumour (MM46) cells inoculated in a C3H/He mice. As a result, the survival of the lectin-treated groups significantly increased, and the treated mice became resistant for tumour cells, showing anticancer activity. Therefore, this activity was accredited lectin-dependent macrophage-mediated cytolysis of cancer cells. On the basis of morphological and biological features such as fragmentation of chromosomal DNA, cell shrinkage, blebbing of plasma membrane, phosphatidylserine externalization, caspase activation and nuclear condensation, the apoptotic cells are characterized. From catfish eggs (*Silurus asotus*), RBL lectins are obtained and bound with glycolsphingolipid-enriched micro domain of Burkitt's lymphoma cells; this as a result induces phosphatidylserine externalization and includes cell shrinkage (Kawano et al. 2009). CSL lectins show better cytotoxic effects against human colon cancer cells by an apoptotic pathway through cell surface recognition in a dose-dependent manner (Shirai et al. 2009). Delivery of target therapeutic molecules to specific sites by drug delivery systems provides various advantages over traditional therapeutics (Bies et al. 2004).

Examination of lectins is done in order to understand the antiproliferative mechanism against cancer cell lines, as a result it shows that the lectins have higher anticancer against the HeLa cell lines. A rhamnose-binding lectin (RBL) was obtained from Chinook salmon roe, which has cytotoxic effects against the human breast cancer cell line (MCF-7) and hepatoma Hep G2 cells (Bah et al. 2011). Likewise in bighead carp, a lectin was isolated from the gills which showed high anticancer activity against the HeLa cell line with antiproliferation action. CGL-type lectins were extracted from a marine mollusc (*Crenomytilus grayanus*); through further study, it is understood that these lectins have the tendency to increase the cell viability and also binding them with surface bound globotriose (Gb3) results in the termination human breast cancer cells (Fig. 18.5).

The targeted drug delivery system (DDS) helps to deliver the drug to specific sites without causing any harm to normal and healthy cells, which as result improves the efficacy of the treatment. The experimental and human tumours shows high levels of N-linked-1,6-GlcNAc oligosaccharides, but the N-glycan has the probable moiety for lectin targeting anticancer drug delivery system. By two mechanisms, the cytotoxicity of DDS based on lectins might be exploited. Out of two mechanisms, one would be a non-toxic lectin that conjugates to a drug, which when reaches the target cell will become toxic during activation. The other mechanism would engage as a toxic lectin with a function as homing moiety and by apoptosis induction.

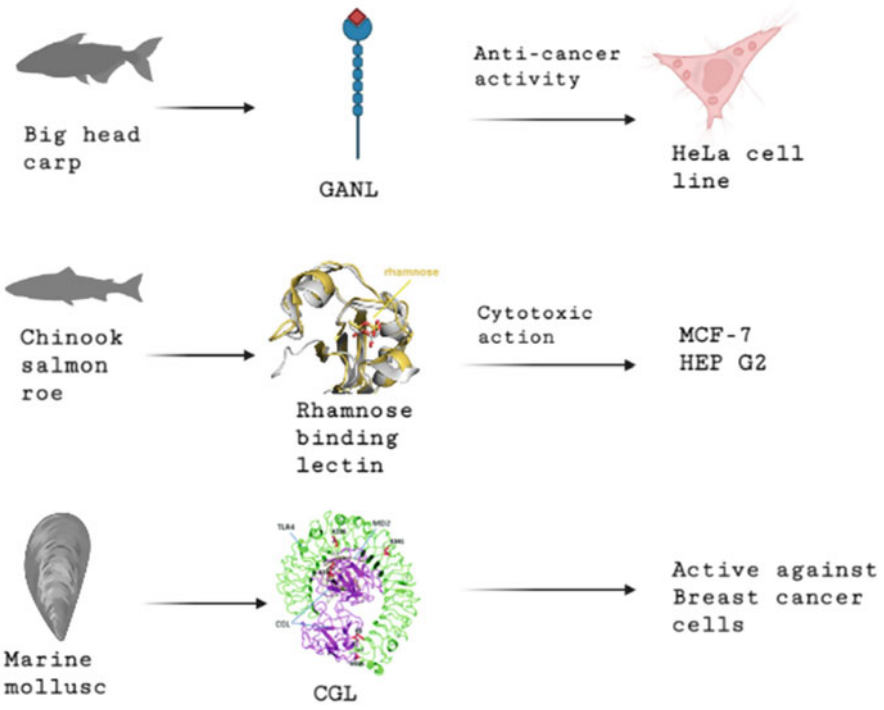

Fig. 18.5 Lectins acting as anticancer agents

18.4.3 Lectins as Antiviral Agents

The Galactose-specific lectins extracted from the sea worm *Chaetopterus variopedatus* and the marine sponge *Chondrilla nucula* have the tendency to inhibit the cytopathic effect of human immunodeficiency virus (HIV-1) and the production of p24 viral antigen by stimulating the oligodenylate metabolism and act as an antiviral agent by blocking the viral entry into the host cells. A specific lectin is isolated from the sea worm *Serpula vermicularis* (Annelida) named GlcNAc lectin; this has the ability to block the viral entry by inhibiting the production of p24 viral antigen with an anti-HIV action followed by a cytopathic effect of HIV-1 at the half-maximal effective concentrations (EC50) of 0.23 and 0.15 μg/mL, respectively (Molchanova et al. 2007). The mannose-specific lectin concanavalin A and Galanthus nivalis agglutinin (GNA) target the surface envelope proteins (glycan shield) of HIV which results in the inhibition of membrane infection and fusion. But these lectins show comparatively low micromolar range of EC50 values during whole-cell anti-HIV assay. On the contrary, high mannose-binding cyanobacterial (Blue-green algae) lectins have higher tendency to inhibit HIV replication with low nanomolecular to picomolecular range of EC50 values. The lectin griffithsin isolated from *Griffithsia* sp. has higher potential to inhibit HIV replication/multiplication

with activity displayed in picomolar concentrations (Mori et al. 2005). In *Boodlea coacta*, a BCA lectin is isolated, and it has three internal tandem-repeated domains with sequence motifs similar to that of GNA-related lectins (Sato et al. 2011). Likewise, BCA lectins exhibit anti-influenza activity against various strains such as isolates of H1N1 pandemic strain (2009) by binding with the viral envelope, haemagglutinin. The prokaryotic lectins are similar to eukaryotic lectins in terms of internal amplification of amino acid sequences along with structural features, but it has no sequence homology. 3D structures of these lectins generally demonstrate domain swapping. Binding the high mannose oligosaccharides on the surface of the glycoprotein gp120 viral envelope with Cyanovirin-N (CV-N) inhibits the HIV infection (Boyd et al. 1997). It also inhibits several other enveloped viruses such as Ebola, herpes virus, influenza and hepatitis C. Each and every CV-N contains two symmetrically related domain structures (A and B); both the domains consist of carbohydrate-binding site that uniquely interacts with α-oligomannose moieties of Man-8 and Man-9 glycans. CV-N stabilization helps in the vital production of recombinants and applied in biomedical field as medical agent. When compared with the wild-type CV-N, it has two CV-N molecules that are kinked in a head-tail fashion with an improved HIV-1 neutralization up to 18-folds (Keeffe et al. 2011). The stabilized domain-swapped dimer shows more potential than anti-HIV antibody neutralization followed by extensive cross-clade reactivity. By substituting the buried polar side chains with aliphatic groups, the CV-N variants are designed to stabilize even against chemical and thermal denaturation. While maintaining higher affinity, it has the tendency to select targeted glycans (Patsalo et al. 2011). Higher yield of increased stability can be obtained by the removal of buried polar groups. Likewise, many anti-viral applications are implied by means of lectins against many viral diseases in human as well as in other organisms.

18.4.4 Lectins as Supplement for Micro Floral Growth in Fishes

Lectins play a vital role in maintaining the microflora of the skin, gut, mucosa, etc. of fish. Acquiring the lectins from the fish system could yield information about the fish microflora. Lectins present in the fish system are non-toxic to the microflora, meanwhile it is toxic to foreign microbes. It is essential in maintaining the microbial count and nutrition supply for the microflora because these microbes purely depend on the nutrition from fish. Saccharides are the only carbon source for microflora. The microbes cannot use all the saccharides from the fish since lectins conjugate with it. Hence, it indirectly governs the nutrition available for the microbes. The epidermal structure of fish accommodates lectins, agglutinins, lysozymes and immunoglobulins. These structures are essential to maintain the microflora. Microflora of one microbiota could be an infectious agent for another. Implementing the lectin of one fish to another species has a higher probability to improve the fish immunity. If it

is possible, then sustainable fish production will also be increased, and it would be the scale-up tool for industries to sustainable products for improving the fish immunity.

18.5 Fish Lectin Research Based on 'Omics' Techniques

Fish lectins that are isolated from various marine species have been hugely increased from the past two decades. When it comes to fish lectins, there are wide varieties of lectins based on its structural and functional characteristics and also include unique carbohydrate specificities. The isolation, identification and physicochemical characterization of lectins are increasing in an exponential way. The functional feature identification of lectins involves validation of their significance of fish diseases and more importantly its downstream effect. Lectins play a major role in fish innate immunity by activating the complement pathway. Due to lectin gene mutations, the functional impairment of specific lectins causes the susceptibility of species or resistant towards various infectious fish diseases. In functional aspects, the molecular mechanisms of fish lectins in the fish immunity are less known, but except for its expression in tissues or organs that are significant to immune functions, binding capacity to prospective pathogens and their infection challenge of up- or downregulation. However, still there is a void for information regarding the regulatory and mechanistic aspects of fish lectins when compared to the studies regarding the mammals (Fig. 18.6).

It is expected that in the near future, the current genomic and transcriptomic approaches on fish models will give an inclusive knowledge about the role of fish lectins as regulators of adaptive immune responses at molecular level or recognition and effectors molecules in innate immunity. Recent research of fish lectins regarding its various characteristics based on the information obtained through the omics data is emerging rapidly (Brinchmann 2016). The advancement in the high-throughput 'omics' techniques such as proteomics, genomics, metabolomics and transcriptomics allows the identification and simultaneous study of various genes and molecules, so this has the ability to discover the unidentified lectin molecules and its functions (Salinas and Magadán 2017). The study of genes from the fish lectins can be facilitated by the development in the field of genomics which also simultaneously helps in the widespread of microbiomics; microbiomics is a study related to the characterization of microbiome (Ao et al. 2015). In European eel, the evolution of aquatic mucosal pathogen occurs due to the role of fish lectin surfaces and acts as a natural niche for the evolution, this phenomenon or evidence is obtained by understanding the DNA sequences (Carda-Diéguez et al. 2017). Transcriptomics is the study of complete set of RNA transcripts which are obtained or produced by the genome. In fish immunity, the discovery of new genes can be done by using a powerful tool, transcriptome, and it also unveils several important aspects of lectin functions such as microbial pathogenesis, responses of host immune and kinetics of such responses (Micallef et al. 2012). The interpretation of

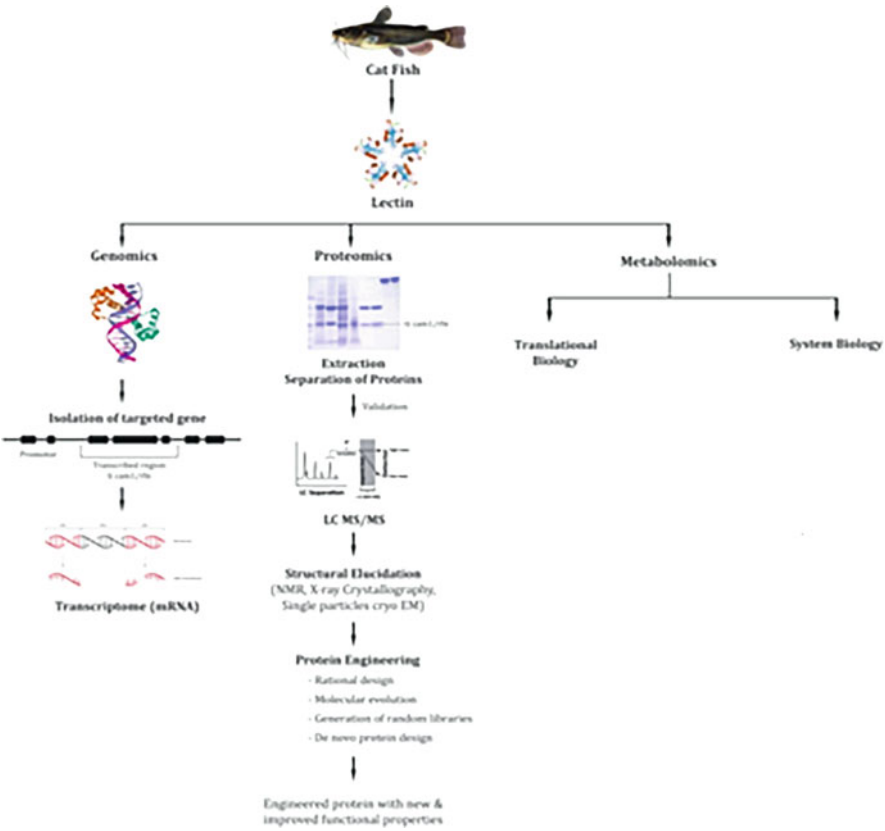

Fig. 18.6 Incorporation of omics technologies in fish lectin

information or data obtained from whole-transcriptome sequencing still remains exigent which depends on well annotated genomes, and also there are fewer data about the non-commercial and uncommon species of fishes. The transcriptomics and gene expression together have been successful in the characterization of immune responses for several fish bacterial infections, especially smoltification in salmon (Kumari et al. 2015)**.** In Atlantic salmon, seven putative lectins with tissue-specific transcription patterns are found through genomic study, and it unveiled those different stressors of aquaculture regulates lectin transcription differently, which as a result provides various insights regarding the fish immunity (Sveen et al. 2017).

In order to understand the fish lectin dynamics, the high-throughput techniques like proteomics helps in the comprehensive comparative study with different types of fishes. Especially these proteomic studies reveal the dynamics of an infected fish, fish exposed with different diets, over population as well as exposure to external stress conditions (Cordero et al. 2015). Likewise, the metabolomics is the study of small molecules in an organism (basically called as metabolome), which provides a clear picture on the actual state of a specific organism under desired conditions at a

specific time. Metabolomics is enormously helpful in understanding the responses of an organism and majorly contributes to the discovery of biomarkers. Since these metabolites are the end products or by-products of regulatory processes, the levels of these metabolites can be regarded as the biological systems ultimate response. The study of these fish lectins can hugely help in the discovery various bioactive molecules and also improves the understanding towards the functions and processes of the above-mentioned fish lectins (Kosmides et al. 2013). Despite of all these information, still there is a void of information about the metabolome of fish lectins and are still unexplored in several aspects. When compared to other omics technologies, the study of fish lectin metabolomics is still exigent because of the accumulation of a large amount of data and lesser amount of information is obtained regarding the fish metabolites and its specialized databases. Therefore, the incorporation of different omics techniques contributes hugely for the advancement in the fish lectin studies/research and exhibits better understanding about fish lectins. The integration of omics technologies paves a path to reveal various functions and physicochemical characters of fish lectins, or else all this information will be unexplored (Buescher and Driggers 2016).

18.6 Conclusion

In the last few decades, the number of lectins extracted from marine resources, particularly fish has risen. In terms of structural and functional features, fish lectins are quite diverse; including carbohydrate specificities are unique and specific. The number of lectins being identified, isolated and physico chemically characterized is growing at a rapid rate. Lectins are major and vital components for stimulating the innate immune system in fish, and they play an important role in innate immunity induction, particularly in the activation of the complement pathway. The functional impairment of specific lectins caused by lectin gene mutations is likely the source of species susceptibility or resistance to many infectious fish diseases, but little is known about the molecular mechanisms of functional features of lectins in fish immunity, except from their expression in immune-related tissues or organs and their down- or upregulation by infectious challenges. Furthermore, when compared to other, knowledge on mechanistic and regulatory features of fish lectins has received less attention, in terms of economic benefits; increasing production of disease-resistant fish would be very appealing to the aquaculture industry. Current genomic and transcriptomic methodologies on fish models are expected to provide comprehensive understanding about their immune responses at the molecular level in the near future.

Conflict of Interest The authors have no conflicts of interest to declare.

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