

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Biological Applications of Plants and Algae Lectins: An Overview

Edson Holanda Teixeira, Francisco Vassiliepe Sousa Arruda,
Kyria Santiago do Nascimento, Victor Alves Carneiro, Celso Shiniti Nagano,
Bruno Rocha da Silva, Alexandre Holanda Sampaio and Benildo Sousa Cavada

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/50632>

1. Introduction

More than 120 years ago, Peter Hermann Stillmark in his doctoral thesis presented in 1888 to the University of Dorpat, gave the earliest step in the study of proteins that have a very interesting feature: the ability to agglutinate erythrocytes. These proteins were initially referred as to hemagglutinins or phytoagglutinins, since they were originally isolated from extracts of plants [1]. The first hemagglutinin isolated by Stillmark was extracted from seeds of the castor tree (*Ricinus communis*) and was named ricin [2]. This hemagglutinin was strongly used by Paul Ehrlich as model antigens for immunological studies [2,3].

Thirty-one years after Stillmark, James B. Sumner, isolated from jack bean (*Canavalia ensiformis*) a protein that he called concanavalin A (ConA). For the first time a pure hemagglutinin had been obtained [4]. However, the report that ConA agglutinates cells such as erythrocytes and yeasts and also precipitates glycogen from solution was given by Summer and Howell nearly two decades after its isolation. In addition, the findings of these researchers showed that the hemagglutination by ConA was inhibited by sucrose, demonstrating for the first time the sugar specificity of lectins. Thus, they suggested that the hemagglutination induced by ConA might be due to the reaction of the plant protein with carbohydrates expressed on the surface of the erythrocytes [1,4].

In 1907, Landsteiner and Raubitschek analyzed the hemagglutination of red blood cells from different animals by various seeds extracts. They found that the relative hemagglutinating activity was quite different for each extract tested [1]. However, it was only in the 1940s that Willian Boyd and Karl Renkonen, working independently, made the important discovery of human blood groups specificity for hemagglutinins. They found that crude extracts of two leguminous plants, *Phaseolus limensis* and *Vicia cracca*, agglutinated blood type A

erythrocytes but not blood type B or O cells, whereas the extract of *Lotus tetragonolobus* agglutinated only blood type O erythrocytes [1,5].

The specific interaction between lectins and carbohydrates of erythrocytes played a crucial role in the investigations of the antigens associated with the ABO blood group system. In the subsequent decade, Morgan and Watkins found that the agglutination of type A erythrocytes by extracts of *Phaseolus limensis* was best inhibited by α -linked N-acetyl-D-galactosamine, while the agglutination of O cells by the extract of *L. tetragonolobus* was best inhibited by α -linked L-fucose [6].

Around thirty years after Boyd, the research on lectins reached the molecular level studies. It was clear the need to a better understanding on the structural aspects of lectins. Then, in 1972 Edelman and colleagues established the primary sequence of ConA [6]. In the same year, Edelman's group and independently Karl Hardman with Clinton Ainsworth, solved the 3D structure of ConA by X-ray crystallography [7,8].

2. What exactly is a lectin?

In 1954 Boyd and Shapleigh proposed the term lectin, from the Latin verb *legere* (which means "to select"). This term was based on the fact that these proteins have the ability to distinguish between erythrocytes of different blood types [9].

Lectins were early defined as carbohydrate-binding proteins of nonimmune origin that agglutinate cells or as carbohydrate-binding proteins other than antibodies or enzymes. However, these definitions were updated, since some plant enzymes are fusion proteins composed of a carbohydrate-binding and a catalytic domain, for instance, type 2 RIPs, such as ricin and abrin, are fusion products of a catalytically active A-chain (which has the N-glycosidase activity) and a carbohydrate-binding B-chain, both linked by a disulfide bond [10]. Furthermore, there is in nature carbohydrate-binding proteins possessing only one binding site and, therefore, are not capable of precipitating glycoconjugates or agglutinating cells [11].

Thus, in 1995 Peumans and Van Damme proposed the most suitable definition for lectins. According to the "new" definition, all plant proteins that possess at least one noncatalytic domain that binds reversibly to a specific mono- or oligosaccharide are considered as lectins [12,13].

2.1. Plant lectins

Lectins are proteins widely distributed in nature such in microorganisms, plants, animals and humans, acting as mediators of a wide range of biological events that involve the crucial step of protein-carbohydrate recognition, such as cell communication, host defense, fertilization, cell development, parasitic infection, tumor metastasis, inflammation, etc [14-15].

Peumans and Van Damme classified the plant lectins according to their overall structure. *Merolectins* consist exclusively of a single carbohydrate-binding domain (e.g. hevein, a

chitin-binding latex protein isolated from the rubber tree *Hevea brasiliensis*). Since merolectins have a unique carbohydrate-binding site, they are incapable of precipitating glycoconjugates or agglutinating cells. *Hololectins* are also built of carbohydrate-binding domains. However, they contain at least two such domains that are identical or very similar. Because these lectins are di- or multivalent they can agglutinate cells and/or precipitate glycoconjugates. Most plant lectins are hololectins. *Superlectins* are built of at least two carbohydrate-binding domains. Unlike hololectins, these domains are not identical or similar. Thus, superlectins recognize structurally different sugar (e.g. TxLCI, a tulip bulb lectin that recognizes mannose and N-acetyl-galactosamine). *Chimerolectins* are fusion proteins that consist of two different chains, one of them with a remarkable catalytic activity (or another biological activity). RIPs type 2 are examples of chimerolectins [11-12].

The most thoroughly investigated lectins have been isolated from plants, particularly that extracted from members of the Leguminosae family. Legume lectins are a large group of proteins that share a high degree of structural similarity with distinct carbohydrate specificities. The subtribe Diocleinae (Leguminosae) comprises 13 genera, mostly of them from the New World. However, only 3 of these genera (i.e. *Canavalia*, *Cratylia* and *Dioclea*) are considered as the main sources for protein purification [16].

Concerning the biological activity, legume lectins are considered as enigmatic proteins. Despite the phylogenetic proximity as well the high degree of similarity shared between them, they possess different biological activities such as histamine release from rat peritoneal mast cells, lymphocyte proliferation and interferon- γ production, peritoneal macrophage stimulation and inflammatory reaction as well as induction of paw edema and peritoneal cell immigration in rats [16].

2.2. Plant lectins as biotechnological tools

Significant progress has been reached in last years in understanding the crucial roles of lectins in several biological processes [17]. The importance of lectins as biotechnological tools has been established early in the studies involving its biological application. In 1960 a major step in immunology was given in order to determine the role of these proteins on the lymphocytes cell division. It was found that the lectin of the red kidney bean (*Phaseolus vulgaris*), known as phytohemagglutinin (PHA), possesses the ability to stimulate lymphocytes to undergo mitosis [18]. After these findings, many studies have been performed to evaluate the role of lectins on different models involving the immune response and its products, for instance the stimulation of cytokine secretion [19], functional activation of monocytic and macrophage-like cells [20] and ROS production by spleen cells [21].

In addition to immunological studies, recent works have been investigated the influence of lectins in the field of microbiology, since lectins can be considered as valuable tools to verify the role of interaction between the pathogen and carbohydrates present in host cells and its importance to disease development. For instance, it has been proposed that the pathogen *Helicobacter pylori* infects human cells through an interaction involving a lectin [22].

3. Carbohydrates and the neoplastic process

Currently, malignancies are considered a major problem in the public health, especially given their increasing incidence and prevalence rates observed in recent decades. In this context, the malignant tumors, or cancers, account for approximately 7.8 million deaths per year, thus becoming the second greatest cause of death worldwide, only behind the cardiovascular disease [23].

The cancer can be defined as a set of more than 100 diseases that have in common the uncontrolled growth of cells, which invade tissues and organs and can spread to other body regions [23]. Thus, both the processes of cellular mutation that affects the neoplastic cell and metastasis, involve a series of genetic changes that culminate in modifications in the pattern of several receptors and signaling molecules present on the cell surface [24].

Carbohydrates are biomolecules that have enormous potential for encoding biological information. These combined-molecules (Glycoproteins and Glycolipids) are responsible for different biological interactions between the cell and the extracellular environment [26]. Regarding the neoplastic cells, the glycosylation of these proteins and lipids is changed, which generates membrane signaling molecules capable of inducing several processes directly related to tumor progression such as cell adhesion, angiogenesis, cellular mitosis and metastasis, in addition, in some cases, be responsible for inhibition of apoptosis induction triggered by the cells of the immune system [26].

Certain changes in glycans occur frequently in neoplastic cells and may be considered "tumor-specific", establishing a correlation between the stage of disease progression and prognosis of the same [25]. Some classic examples of these changes are the antigens of the ABH and Lewis system. ABH antigens are not expressed in cells of healthy human colon but significantly expressed on tumor cells [27]. Since the antigen Lewis^y can be observed in several carcinomas and has been correlated with poor prognosis in breast tumors [28]. Another example is the glycosylated antigens sialyl-Lewis^A and sialyl-Lewis^x, which are significantly up-regulated in carcinomas of the colon and appear to be related to tumor progression [29].

Thus, due to the intrinsic role of carbohydrates in the tumorigenesis, the glycosylation process as well as the identification of glycosylated antigens have been intensively focused, given the fact that glycosylation of antigens can vary extensively depending on the stage of the disease, which can provide, when properly identified, a better possibility of correct diagnosis and treatment.

4. Application of plant lectins in the diagnosis of malignant tumors

Because the peculiar characteristic of specific binding to carbohydrates, lectins have been used as tools to identify aberrant glycans expressed by neoplastic cells. Such methods have been essential to obtain a more precise diagnosis that allows a more accurate prognosis.

Several methods regarding the use of lectins as tools for recognizing aberrant glycans have been proposed in recent days [30,31,32]. The technique most common and widespread is the use of lectins in immunohistochemical assays.

In this context, the study conducted by [30], showed that leguminous lectins from *Canavalia ensiformis* (ConA) and *Ulex europaeus* (UEA-1) were used as histochemical markers of parotid gland mucoepidermoid carcinoma with low, intermediate, and high grade dysplasia. The authors stated that ConA localization in the cytoplasm and/or plasma membrane was significantly associated with neoplastic cells from the three grades of severity, whereas UEA-1 was associated with low and intermediate grade dysplasia. The authors obtained similar results after the analysis of other cell regions.

Another recent methodology was addressed by [33]. This methodology exploits the fact that glycoproteins produced by cancer cells have altered glycan structures, although the proteins themselves are common, ubiquitous, abundant, and familiar. However, as cancer tissue at the early stage probably constitutes less than 1% of the normal tissue in the relevant organ, only 1% of the relevant glycoproteins in the serum should have altered glycan structures [34]. With that in mind, the strategy to approach the detection of these low-level glycoproteins is based in: (a) a quantitative real-time PCR array for glycogenes to predict the glycan structures of secreted glycoproteins; (b) analysis by lectin microarray to select lectins that distinguish cancer-related glycan structures on secreted glycoproteins; and (c) an isotope-coded glycosylation site-specific tagging high-throughput method to identify carrier proteins with the specific lectin epitope [33].

Therefore, further analyses of lectins as biomarkers have been undertaken to improve our understanding of the processes involved in malignant tumor formation. As well as enable us to acquire new methods of identification of neoplastic cells at an early stage, enabling a better prognosis with appropriate treatment and low cost.

5. Application of plant lectins in the treatment of malignant tumors

Apoptosis is a mechanism by which cells undergo death to control cell proliferation or in response to irreparable DNA damage. It is featured by unique morphological and biochemical changes, such as nucleus condensation and margination, membrane blebbing, and internucleosomal DNA cleavage [35]. As the type I programmed cell death (PCD), apoptosis occurs through two major pathways, the extrinsic pathway triggered by the Fas death receptors, and the mitochondria-dependent pathway that brings about the release of Cytochrome *c* (Cyto *c*) and activation of the death signals under stimulus. In both ways the caspases, which belong to a family of cysteine proteases, have been well established as major players in apoptosis-causing mechanisms [36].

Several studies have demonstrated that lectins can induce apoptotic cell death. In the mitochondrial-dependent pathway, ConA treatment results in a decrease of mitochondrial membrane potential, and thus collapsing mitochondrial transmembrane potential. Cyto *c* is subsequently released, making up apoptosome with Apaf-1 and procaspase-9. After

conjugating apoptosome, procaspase-3 turns into active caspase-3 that eventually triggers apoptosis [37].

In [38], it was evaluated the pro-apoptotic activity of a lectin isolated from *Artocarpus incisa* (frutalin) on HeLa cells derived from human cervical cancer. In this study, frutalin possessed a remarkable antiproliferative effect on HeLa cells. This effect was irreversible as well as time and dose dependent. When the lectins were added, serious visible cellular morphology changes were observed, possibly as a result of cell stress.

An interesting study conducted by [39] showed the pro-apoptotic caspase-dependent activity of the lectin isolated from *Astragalus membranaceus* in leukemia cell line (CML K562). These results showed a close relationship with the low expression of BCL-2 (anti-apoptotic protein) indicating that the lectin is active through the mitochondrial apoptotic pathway [40]. Furthermore, structural changes in cell membrane and different levels of Caspase-3 contributed to support their hypothesis.

Despite the apoptotic activity of several lectins have been demonstrated in different studies, the precise mechanism of how this process is triggered as well the mode of internalization is unknown until the present date.

6. Carbohydrates and the inflammatory process

The inflammation is a nonspecific event of immune response that occurs in reaction to any type of tissue injury. This process is capable of triggering a series of physiological changes such as increased blood flow, elevated cellular metabolism, vasodilation, release of soluble mediators, extravasation of fluids and cellular influx [41].

The continuity of cell recruitment and tissue damage in addition to chemical mediators released by the injured tissue as well as resident cells on site activate various mechanisms, in turn, induce the migration of more immune cells as well as increasing local tissue perfusion [42].

Both, acute and chronic inflammations have specific characteristics and the innate immune system plays a central role, since it mediates the initial response. Infiltration of innate immune cells, specifically neutrophils and macrophages, characterizes the acute inflammation, while infiltration of T lymphocytes and plasma cells are features of chronic inflammation [41,42].

As discussed previously, carbohydrates can act as the intermediates of communication in biological processes such as differentiation, proliferation and certain cell–cell interactions that are crucially important in both physiological and pathological phenomena [43,44]. The information contained in the enormous variety of oligosaccharide structures normally conjugated to proteins or lipids on cell surfaces (glycocodes) is recognized and deciphered by a specialized group of structurally diverse proteins, the lectins [44].

Galectins (formerly “S-type lectins”), an evolutionarily conserved family of endogenous animal lectins, share unique features, including their highly conserved structure, exquisite

carbohydrate specificity, and ability to differentially regulate a myriad of biological responses [45].

Although galectins have been implicated in many biological activities, most of the functional studies reported to date link galectins to early developmental processes, such as neovascularization, regulation of immune cell homeostasis and inflammation [44,46,47]. Through deciphering glycan-containing information about host immune cells or microbial structures, galectins can modulate a diversity of signaling events that lead to cellular proliferation, survival, chemotaxis, trafficking, cytokine secretion and cell-cell communication [46,47].

These findings are extremely important because they demonstrate the importance of the glycodes in the process of cell recognition and inflammation. In this context, plant lectins have been widely used to understand the pro-inflammatory mechanisms, as well as the design of new compounds with pro-healing effect.

7. Plant lectins and its immunomodulatory activity

An immune system is a system of biological structures and processes within an organism that protects against disease. In order to function properly, an immune system must detect a wide variety of agents, from viruses to cancer cells, and distinguish them from the organism's own healthy tissue [41]. The immune system is composed of many cells and molecules that act in a complex and harmonious way with the ultimate goal of annihilating the aggressive factor [42].

In the immune system, two phases of activity can be clearly established: the innate immune response and the adaptive immune response. In the innate immune response, there is the activity of cells and cytokines in a nonspecific way with the main purpose to annihilate quickly the local damaging agents. At this stage, we highlight the neutrophils, eosinophils, basophils and macrophages, cells with well-established activities but with the common function of production and release of cytokines. These cytokines are molecules with various functions in the inflammatory process, such as chemotaxis, activation of certain cell groups and increased tissue perfusion [48].

On the other hand, the adaptive immune response is composed by another set of cells that acts in a more specific way, the lymphocytes. Such cells are responsible for producing antibodies specific for certain invading microorganisms and the activation of mechanisms of apoptosis in abnormal cells [49].

Thus, the use of molecules capable of inducing cell recruitment as well as cytokine production and lymphocytes proliferation is of special scientific interest.

Korean mistletoe (*Viscum album* L. var. *coloratum*) is traditionally used as a sedative, analgesic, anti-spasmodic, cardiogenic and anticancer agent, in Korea. An important lectin has been isolated from this plant and its immunomodulatory activity was analyzed [50]. It was shown that KML differentially modulated macrophage-mediated immune responses. It

also enhanced the expression of various cytokines (IL-3, IL-23 and TNF- α), ROS generation, phagocytic uptake and surface levels of some glycoproteins (co-stimulatory molecules, PRRs and adhesion molecules). Nevertheless, the functional activation of adhesion molecules assessed by cell–cell or fibronectin adhesion events was up-regulated by KML treatment.

A recent study [51] demonstrated the immunomodulatory activity of ConBr, a lectin isolated from *Canavalia brasiliensis* seeds. The assays showed that ConBr was able to induce *in vitro* proliferation of splenocytes with minimal damage to the cellular structure. Furthermore, ConBr increased in the production of cytokines such as IL-2, IL-6 and IFN- γ production and decreased IL-10. These findings indicate the potential immunomodulatory effect of this lectin in conjunction with the intrinsic role of carbohydrates in intercellular communication related to the inflammatory process.

Regarding the activity of lectins on lymphocytes, a recent study [52] evaluated the effect of lectin extracted from seeds of *Cratylia mollis* Mart. (Cramoll 1,4) on experimental cultures of mice lymphocytes. In this study, aspects directly related to inflammation as cytokine production, cytotoxicity and cellular production of nitric oxide (NO) were evaluated. Cramoll 1,4 did not show cytotoxicity at the concentrations tested, in addition, was able to induce IFN- γ and showed an anti-inflammatory activity through the suppression of NO production.

The biological function of carbohydrates in inflammation events is well-defined. In this context, proteins that bind specifically to such glycans are of great interest because of their possible functions and applications in biotechnological studies.

8. Plant lectins and its pro-healing activity

Recently, researches have undertaken efforts at the possible pro-healing activity of some lectins [53,54]. This goal is supported by the fact that such molecules may interfere with the inflammatory process. This effect is not yet fully elucidated, however peculiar and interesting results can be observed.

Experiment conducted in a murine model, employing the lectin isolated from *Bauhinia variegata* seeds (BVL) topically on surgically induced wounds, revealed the pro-healing potential of such molecule. Although not yet elucidated, it is suggested that this lectin appears to stimulate the mitogenic activity of resident cells, turning them into potent chemotactic agents for the recruitment of neutrophils through the release of cytokines [54]. Furthermore, it is suggested that the BVL stimulates the differentiation of fibroblasts into myofibroblast, which is an extremely important event in the remodeling of connective tissue [55].

Although promising, this issue requires further studies to better characterize the mechanisms involved in pro-healing role played by lectins.

9. Marine algae lectins

To date, there are fewer than 100 publications describing the presence of lectins in marine red, green and brown macroalgae. Moreover, and in marked contrast to higher land plant

lectins, marine algal lectins have been isolated and characterized at a much lower pace since the first report of haemagglutinating activity in these organisms appeared more than 46 years ago [56]. Thereafter, other studies describing the presence and/or purification of algal lectins were reported by groups from England [57], Japan [58], Spain [59], United States [60] and Brazil [61].

Currently, the presence of lectins was analyzed at about 800 algae species. However, this number is still small, considering that there are thousands of species of marine algae. Together, the research shows that approximately 60% of the analyzed species show hemagglutinating activity. The number of positive species could be higher since in the first screenings the authors used a limited number of red blood cells and without enzyme treated erythrocytes.

The improvement in the methodologies of both, extraction and hemagglutination assays could increase the number of positive species. In fact, there appears to be coincidence that the rabbit erythrocytes treated with papain are most suited for the hemagglutinating activity detection in marine macroalgae [62,63].

Although marine algal lectins show proteinaceous content similar to lectins from terrestrial plants, they differ in some aspects. Early publications on this issue, reported that in general, lectins from algae have low molecular masses, no affinity for monosaccharides, strong specificity for complex oligosaccharides and/or glycoproteins. Moreover, they appear to have no requirement for metal ions, showing high content of acidic residues and even in high concentrations tend to stay in the monomeric form [64,65,66]. However there are a few reports showing that some of these molecules may be inhibited by simple sugars and are cation dependent as showed for the lectins from the green marine alga genus, *Codium* [67] and red marine alga genus, *Ptilota* [68,69,70].

The classical methods used to purify marine algae lectins include methods such as protein precipitation (using salt or ethanol), liquid chromatography (especially affinity) and electrophoresis [69,71]. Ion exchange chromatography has been effectively used in the isolation of lectins from seaweed, mainly in initial stages in purification. In this step, the lectins were separated from pigments present in the extracts [66,72,73]. In the protein extracts, phycobilins are co-extracted with lectins, becoming an undesirable contaminant in the purification process [72].

Lectins from marine algae *Cystoclonium purpureum* [74], *Gracilaria verrucosa* [75], *Palmaria palmata* [76], *Solieria robusta* [62], *Gracilaria tikvahiae* [60], *Bryothamnion seaforthii* and *B.triquetrum* [66], *Solieria filiformis* [77], *Enantiocladia duperreyi* [78], *Amansia multifida* [73], *Hypnea musciformis* [79], *Gracilaria ornata* [80], *Hypnea cervicornis* [81] and *Georgiella confluens* [82] were isolated by exchange chromatography, usually on DEAE cellulose. In contrast, due usually algal lectins have binding specificity of complex sugars, affinity chromatography has been used a few times, such as the lectins of green algae of the genus *Codium* [83,84,85], lectins from *Ulva lactuca* [86], *Caulerpa cupressoides* [87], *Enteromorpha prolifera* [88], *Ulva*

pertusa [89], *Bryopsis plumosa* [90,91,92], *Bryopsis hypnoides* [93] and lectins from red marine algae of the genus *Ptilota* [68,69,70].

To date, only 31 lectins from Rhodophyceae and 17 lectins from Chlorophyceae were isolated and characterized. The virtual absence of lectins isolated from brown algae (Phaeophyta) is mainly due to the amount of polyphenols present in plants. It is known that polyphenols are released in extraction and that these compounds and their oxidation products, quinones, bind tightly to proteins [94] causing a false hemagglutination [83,95].

Even with the increase in the publications related to marine algae lectins, biochemical and structural information on algal lectins is scarce and from only a few species, and hence the functional and phylogenetic classification of these lectins remains unclear. The available structural information indicates the existence of different carbohydrate-binding proteins in the marine algae investigated. Moreover, the complete amino acid sequences of only 14 algal lectins have been determined. In red marine algae, *Bryothamnion triquetrum* lectin (BTL) was the first lectin to be determined its primary structure [96].

In same year, [97] reported the primary structure of *Hypnea japonica* agglutinin (HJA) that shares sequence similarity with BTL and with lectin from *Bryothamnion seaforthii* [98] and these lectins constitute the first marine red alga lectin family. Based on identity between HML and HCA and in the differences in amino acid sequences compared with BTL/HJA, [99] suggest that HCA/HML constitute another algal lectin family.

On the other hand, the lectins isolated from *Eucheuma serra*, *E. amakusaensis*, *E. cottonii* [100] have masses around 28 kDa, presents a monomer, share N-terminal sequence similarity with the complete amino acid sequence of isolectin 2 from *Eucheuma serra* (ESA-2) [101]. Also, the primary structure of ESA-2 contains repeated domains in their primary structure. These data suggest that lectins from genus *Eucheuma* can be grouped in a thirty family of red marine alga haemagglutinins. The amino acid sequence of lectin from red marine alga *Griffithsia* sp. [102] displays sequence similarity with lectin from jack fruit (*Artocarpus integrifolia*). The common methodology employed to determine the primary structures from red marine alga lectins was a combination of Edman degradation of sets of overlapping peptides and mass spectrometry. From green marine algae, the first primary structure determined was the lectin from *Ulva pertusa* (UPL-1) [89]. [103] described a 19 kDa protein expressed in strictly freshwater conditions in species of *Ulva limnetica* Ichihara et Shimada (freshwater alga), and this protein (named ULL) was identified and sequenced by cDNA cloning. The protein encoded by the cDNA showed 30% identity to UPL-1. However, the ULL should be considered a lectin-like since its haemagglutination activity was not yet characterized. UPL-1 and ULL did not show amino acid sequence similarity with known plant and animal lectins.

Recently [90] reported that the aggregation of cell organelles of *Bryopsis plumosa* in seawater was mediated by a lectin–carbohydrate complementary system and the purified lectin (named bryohealin) is involved in protoplast regeneration. The primary structure of bryohealin and of lectin from *Bryopsis hypnoides* [104] had few similarity with any known plant

lectin, but rather resembled animal lectins with fucoselectin domains. In addition, *Bryopsis plumosa*, has other two lectins described.

The lectin BPL-2 is a 17 kDa protein specific to D-mannose (ref). The authors found no similarity with others proteins in specific databases. The BPL-3 [92] possesses specificity to N-acetyl-D-galactosamine/N-acetyl-D-glucosamine and share the same sugar specificity with bryohealin. However, the primary structures of the two lectins were completely different. The homology sequence analysis of BPL-3 showed that it might belong to H lectin group from Roman snails (*Helix pomatia*). The latest primary structure published was of lectin from *Boodlea coacta* (BCA) [105]. BCA consisted of 3 internal tandem-repeated domains, each one containing the sequence motif similar to the carbohydrate-binding site of land plant lectin from *Galanthus nivalis*. It should be noted that the primary structures from green marine algal lectins were mainly determined with combination of Edman degradation and cDNA cloning.

Another observation is that a large number of sequenced algal lectins have the presence of cysteinyl residues or the duplication of internal domain. Still a lot of work needs to be done on the structure of algal lectins, since a few amount of primary structures has been determined. Further structural studies will contribute to understanding the differences in their biochemical characteristics as well as to the evolutionary aspects upon lectin presence in land plants and marine algae.

10. Marine algae lectins and its biotechnological role

There are few studies in the literature about the biotechnological applicability of lectins from marine algae. Probably due to low rentability of lectins obtained through the purification processes. It is noteworthy that the majority of lectins isolated so far were extracted from red algae, which are rich in carbohydrates and not in proteins. Moreover, during the extraction of algae proteins, phycobiliproteins are extracted simultaneously, and therefore the addition of steps to remove these phycobiliproteins causes losses of other proteins, among these are the lectins.

However, several studies on biological applications of algae lectins demonstrate that these molecules have an additional benefit; they are molecules with low molecular weight and may be less antigenic when used in biological models.

In cancerology, it was demonstrated that a lectin from the red marine algal *Eucheuma serra* (ESA) induced cell death in human cancer cells through the induction caspase-3 activity and DNA fragmentation in human colon adenocarcinoma (Colo201) cells [106]. ESA also induced cell death in a dose-dependent manner via apoptosis pathway in *in vitro* studies with Colon adenocarcinoma (Colon26) Cells derived from BALB/c mice [107].

In current studies, Span 80 vesicles (a potential type of nonionic vesicular drug delivery system) with ESA and PEGylated (EPV) lipids immobilized, showed hemagglutinating activity similar to free ESA and decreased the viability of Colo201 cancer cells *in vitro* and not

affected the growth of normal cells. EPV caused the anti-tumor effect *in vivo* by inducing apoptosis in tumor cells [108].

Bryothamnion seaforthii lectin (BSL) and *Bryothamnion triquetrum* lectin (BTL) were able to differentiate human colon carcinoma cell variants. Differentiation was, probably, in function to cell membrane glycoreceptors and could be exploited to investigate structural modification of cell membrane glycoconjugates in cancer cell systems. In addition, it has been shown that the binding of both lectins to the carcinoma cells results in their internalization, which is a very interesting property that could be used in future applications, such as drug delivery [109].

The lectins isolated from the red marine algae *Hypnea cervicornis* (HCA), *Pterocladia capillacea* (PcL) and *Caulerpa cupressoides* (CcL) have been tested as anti-inflammatory and antinociceptive agents. The data indicated that HCA, PcL and CcL have actions anti-inflammatory and antinociceptive (in formalin and acetic acid models). However, these lectins did not present significant antinociceptive effects in the hot plate test [110,111,112].

Concerning the mitogenic activity, the lectin from the red marine alga *Carpopeltis flabellate* (Carnin) was the first lectin that showed mitogenic activity for T lymphocytes from mouse spleen at a concentration of 10,5 µg/ml. Carnin inhibited the normal embryonic development of the sea urchin *Hemicentrotus pulcherrimus* at the stage of blastula and also inhibited the gastrulation induced in starfish *Asterina pectinifera*, at concentrations of 10 and 5 µg/mL, respectively. In addition, it was showed an interaction between a lectin of macroalgae with a microorganism of the marine ecosystem [113].

The antibacterial effect was too evaluated. The lectins from the red marine algal *Eucheuma serra* (ESA) and *Galaxaura marginata* (GMA) have an antibiotic activity. ESA and GMA strongly inhibited *Vibrio vulnificus*, a fish pathogen, but not were able to inactivate *V. neresis* and *V. pelagius* [114]. BSL and BTL also were able to avoid the bacterial adhesion of streptococci strains in enamel pellicles. BTL was more efficient in avoiding the adherence of *Streptococcus sobrinus* and *S. mitis* and BSL was able to reduce adherence of *S. mutans*. This fact can contribute with preventing caries at early stages [115].

Red marine algae lectins from *Amansia multifida* (AML), *Bryothamnion seaforthii* (BSL), *Bryothamnion triquetrum* (BTL) and *Gracilaria caudata* (GCL) induced neutrophil migration *in vitro* and *in vivo* in the peritoneal cavity or dorsal air pouch of rats or mice. The results showed that BT had the most potent effect in neutrophil migration when tested on rats. In mice, BS required four times higher than the dose of BT to induce neutrophil migration. However, when the algae lectins were assayed in mice, AML was the most potent [116].

Concerning the antiviral effects, a lectin named KAA-2 from the red marine algal *Kappaphycus alvarezii* (specific to high mannose type N-glycans) showed antiviral action against H1N1 virus [117]. The red alga lectin from *Griffithsia* sp. called griffithsin (GRFT) is a small mannose specific lectin that binds carbohydrates on HIV envelope glycoproteins and block HIV entry into target cells. GRFT is a candidate for development of anti-HIV microbicides [118,119]. GRFT was also able to inhibit the action of the

pathogenic agent responsible for respiratory syndrome (SARS), the SARS coronavirus (SARS-CoV) [120].

11. Lectins as biotechnological tools to study the microbial biofilms

Biofilms are microbial complex communities established in a wide variety of surfaces and are generally associated with an extracellular matrix composed by several types of polymers [121]. This type of microbial association can develop on biotic and abiotic surfaces, including living tissues, medical devices and/or industrial water piping systems and marine environments [122,123,124].

Bacterial infections involving biofilm formation are usually chronic and often present a arduous treatment [125,126,127]. The growth and proliferation of microorganisms inside the biofilm provides reduction or prevents the penetration of various antimicrobial agents [128,129] and thus become extremely difficult or impossible to eradicate them [130,131,132]. For some antibiotics, the concentration required to eliminate the biofilm can be up to a thousand times higher than required to planktonic form of the same specie [127].

The ability of microorganisms to form pathogenic cell aggregates is a worldwide concern. In attempt to remedy this problem, pharmaceutical companies associated to research groups work avidly to the development of new options for the treatment of infections caused by bacteria organized in biofilms.

Biofilm formation is a process in which bacteria has a change in lifestyle, it goes from a state unicellular in suspension to a multicellular sessile, where the growth and cell differentiation results in structured communities. The biofilm begins with the setting free of microorganisms in a given area. The first microorganisms adhere initially by weak interactions, mainly of the van der Waals forces [133]. If the colonies are not immediately removed from the surface, they can anchor by cell adhesion molecules existing in the pili and / or flagella [134]. The first colonies facilitate the arrival of other cells through adhesion sites and begin to build the matrix that will form the biofilm. Only a few species are able to adhere to a surface *per se*. Others may anchor to the matrix or directly on existing colonies. Since the colonization has started, the biofilm grows through cell division and combination of the recruitment of other cells [135].

According to [136], the biofilm development occurs through three events. At first, there is a distribution of fixed cells in surface through cell motility. Then, occurs the proliferation of fixed cells by division, expanding to upward and sides forming agglomerated of cells, similar to the formation of colonies on agar plates [137]. Finally, clusters of cells attached to the biofilm are recruited by the action of the environment itself to the development of other biofilms, reaching a climax community [136]. These general stages provide guidance to the study of biofilms by bacteria furniture, although many details of regulation of this process may vary between species.

One of the biofilm-forming components of great importance in the maintenance of cell clusters is the matrix polymeric called EPS (*Extracellular Polymeric Substances*) [138].

Consisting of proteins, polysaccharides and environmental waste results in a solid structure highly hydrated with small channels between the microcolonies [139]. This matrix holds the biofilm together, one of those factors responsible for providing an increased resistance to antibiotics, disinfectants, and ultraviolet radiation [140]. In most biofilms, microorganisms that make up these agglomerates constitute less than 10% dry weight, while the extracellular matrix may contribute up to 90% [141].

The first step to biofilm formation, the early adhesion, is considered essential for colonization and infection by pathogenic bacteria. Macromolecules surfaces are directly involved in this stage [142]. Proteins known as adhesins are able to recognize specific polysaccharide substrate present on the surface to be colonized, for example, the presence of carbohydrates existing in the film of saliva that covers the teeth in the oral environment [143]. Glycidic epitopes present on surfaces of microorganisms (early colonizers) can also be recognized by these proteins to mediate an event known as coaggregation, which will start the formation of a multi-species community [144].

One etiological factor for the development of teeth biofilms is the adhesion of pathogenic bacteria in the dental enamel [145]. However, the microorganisms are not deposited directly on the tooth surface, but bind to a thin acellular layer composed of salivary proteins and other macromolecules that cover the tooth surfaces called acquired pellicle [146,147].

The acquired pellicle is formed by glycoproteins and carbohydrates that serve as receptors for bacteria containing proteins with glucan-binding domains [148]. Bacteria interact with the film by several specific mechanisms, including the interaction lectin-like involving the bacterial adhesins and receptors existing in the pellicle [148,149]. Next, other bacteria can adhere to the film as well as the bacteria pre-existing in the biofilm [150].

The event coaggregation is a phenomenon widely observed in diverse microbial communities [151,152,153]. Cells can interact in suspension, forming cell aggregates, as well as connect directly on biofilm in the process of formation. In the first case, planktonic cells recognize specifically species genetically distinct creating the coaggregates. In other situations, coaggregates in the form of secondary colonizers can adhere on biofilm in development, a process known as coadhesion. Both cases have an important role in the integration and establishment of a mature biofilm [154].

Thus, carbohydrate residues have an important role in formation and maintainability of microbial biofilms. They act as mediators of the binding between bacteria and the surface that will serve as substrate for biofilm formation [155], as well as site of interaction between microorganisms to form cell aggregates [156,157]. Furthermore, through EPS matrix, maintains the biofilm attached in the surface, conferring a greater resistance to antimicrobial agents in general [158,159].

Molecules able to bind specifically and selectively to carbohydrates have a key importance in the development of research related to microbial biofilms. Thus, lectins have been shown as powerful tools for analyze the glycidic structures of those aggregates from microbial origin [160,161].

Studies of microbial biofilms through the interaction with lectins have two main objectives: visualization and characterization of polymeric matrix (EPS) formed by different species of microorganisms [162,163] and inhibition of oral biofilm formation by blocking the bacterial binding sites present in the pellicle of saliva in the form of glycoproteins and / or carbohydrates [164].

The application of lectins in the characterization of EPS is already widely exploited by many research groups. Lectin of wheat germ (WGA) was used as a marker of *Staphylococcus epidermidis* microcolonies, mainly in the study of the mechanisms involved in bacterial organization during the formation of the cell aggregates [165]. In a study published by the same group, WGA was used to quantify the production of GlcNAc β -1,4*n*, a sugar component of the extracellular matrix involved in biofilm formation [166].

In 1980s was developed a system called ELLA (*Enzyme-linked Lectin Assay*) that uses enzyme-lectin conjugates to detect specific carbohydrate units on the surface of cells. This assay allows better detection and quantitation of the sugars by standard immunofluorescence with fluorescein-conjugated lectins [167]. The lectins concanavalin A (ConA) and WGA peroxidase-labeled were used to detect D-glucose or D-mannose and N-acetyl-D-glucosamine or N-acetyl-neuraminic acid, respectively, on the biofilm of several species [168].

Recent studies demonstrated the characteristics and distribution of glycoconjugates in cyanobacteria biofilm using lectins with different specificities. In this study the authors stated that the distribution of carbohydrates in the matrix is very variable. Based on lectin specificity, glycoconjugates produced by cyanobacteria biofilm contained mainly fucose, N-acetylglucosamine or -galactosamine and sialic acid [169].

Lectins may be a suitable antiadhesion agent for *streptococci*, since it has been reported that a lectin-dependent mechanism is involved in its adhesion [170]. However, the application of lectins as tools to interfere with biofilm formation is still poorly explored [115,171]. As shown by [172], plant lectins appear as a new strategy to reduce the development of dental caries by inhibiting the early adherence and subsequent formation of *Streptococcus mutans* biofilm.

Author details

Edson Holanda Teixeira, Francisco Vassiliepe Sousa Arruda and Bruno Rocha da Silva
LIBS, Integrated Laboratory of Biomolecules, Faculty of Medicine of Sobral, Federal University of Ceará, Fortaleza, CE, Brazil

Kyria Santiago do Nascimento, Victor Alves Carneiro and Benildo Sousa Cavada
BioMol, Laboratory of Biologically Active Molecules, Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza, CE, Brazil

Celso Shiniti Nagano and Alexandre Holanda Sampaio
BioMar, Laboratory of Marine Biotechnology, Department of Fishing Engineering, Federal University of Ceará, Fortaleza, CE, Brazil

Acknowledgement

We would like to thank CNPq, CAPES, FUNCAP and UFC for financial support. AHS, BSC, CSN, EHT, KSN are senior investigators of CNPq.

12. References

- [1] Sharon N, Lis H (2004) History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology*. 14: 53R-62R.
- [2] Franz H (1988) The ricin story. *Adv Lectin Res.* 1: 10-25.
- [3] Olsnes S (2004) The history of ricin, abrin and related toxins. *Toxicon*. 44: 361-70.
- [4] Sumner JB, Howell SF (1936) The identification of the hemagglutinin of the jack bean with concanavalin A. *J Bacteriol.* 32: 227-237.
- [5] Boyd WC, Shapleigh E (1954) Specific precipitation activity of plant agglutinins (lectins). *Science*. 119: 419.
- [6] Morgan WT, Watkins WM (2000) Unraveling the biochemical basis of blood group ABO and Lewis antigenic specificity. *Glycoconj J.* 17: 501-530.
- [7] Edelman GM, Cunningham BA, Reeke GN, Becker JW, Waxdal MJ, Wang JL (1972) The covalent and threedimensional structure of concanavalin A. *Proc Natl Acad Sci USA*. 69: 2580-2584.
- [8] Hardman KD, Ainsworth CF (1972) Structure of concanavalin A at 2.4-Å resolution. *Biochemistry*. 11: 4910-4919.
- [9] Hou FJ, Xu H, Liu WY (2003) Simultaneous existence of cinnamomin (a type II RIP) and small amount of its free A- and B-chain in mature seeds of camphor tree. *Int J Biochem Cell Biol.* 35: 455-64.
- [10] Van Damme EJM, Balzarini J, Smeets K, Van Leuven F, Peunans WJ (1994) The monomeric and dimeric mannose binding proteins from the Orchidaceae species *Listera ovata* and *Epipactis helleborine*: sequence homologies and differences in biological activities. *Glycoconjugate J.* 11: 321-332.
- [11] Peumans WJ, Van Damme EJ (1995) Lectins as plant defense proteins. *Plant Physiol.* 109: 347-52.
- [12] Van Damme EJ, Peumans WJ, Barre A, Rouge P (1998) Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Crit Rev Plant Sci.* 17: 645-662.
- [13] Sharon N, Lis H (1989) Lectins as cell recognition molecules. *Science*. 246: 227-234.
- [14] Sharon N (2007) Carbohydrate-specific reagents and biological recognition molecules. *J Biol Chem.* 282: 2753-2764.
- [15] Cavada BS, Barbosa T, Arruda S, Grangeiro TB, Barral-Netto M (2001) Revisiting *proteus*: do minor changes in lectin structure matter in biological activity? Lessons from and potential biotechnological uses of the Diocleinae subtribe lectins. *Curr Protein Pept Sci.* 2: 123-135.
- [16] Wu AM, Lisowska E, Duk M, Yang Z (2009) Lectins as tools in glycoconjugate research. *Glycoconj J.* 26: 899-913.

- [17] Nowell PC (1960) Phytohemagglutinin: an initiator of mitosis in culture of animal and human leukocytes. *Cancer Res.* 20: 462-466.
- [18] de Oliveira Silva F, das Neves Santos P, de Melo CM, Teixeira EH, de Sousa Cavada B, Arruda FV, Cajazeiras JB, Almeida AC, Pereira VA, Porto AL (2011) Immunostimulatory activity of ConBr: a focus on splenocyte proliferation and proliferative cytokine secretion. *Cell Tissue Res.* 346: 237-44.
- [19] Lee JY, Kim JY, Lee YG, Byeon SE, Kim BH, Rhee MH, Lee A, Kwon M, Hong S, Cho JY (2007) *In vitro* immunoregulatory effects of Korean Mistletoe lectin on functional activation of monocytic and macrophage-like cells. *Biol Pharm Bull* 30: 2043-2051.
- [20] Melo CML, Paim BA, Zecchin KG, Morari J, Chiarrati MR, CorreiaMTS, Coelho LCBB, Paiva PMG (2010) Cramoll 1,4 lectin increases ROS production, calcium levels and cytokine expression in treated spleen cells of rats. *Mol Cell Biochem.* 342: 163-169.
- [21] Ringnér M, Valkonen KH, Wadström T (1994) Binding of vitronectin and plasminogen to *Helicobacter pylori*. *FEMS Immunol Med Microbiol.* 9: 29-34.
- [22] Bennett HJ, Roberts IS (2005) Identification of a new sialic acid-binding protein in *Helicobacter pylori*. *FEMS Immunol Med Microbiol.* 44: 163-9.
- [23] Curado M, Edwards B, Shin H, Storm HH, Ferlay J, Heanue M, Boyle P (2008) Cancer incidence in five continents. Lyon: International Agency for Research on Cancer. 837 p.
- [24] Saeland E, van Kooyk Y (2011) Highly glycosylated tumour antigens: interactions with the immune system. *Biochem Soc Trans.* 39: 388-92.
- [25] Nangia-Makker P, Conklin J, Hogan V, Raz A (2002) Carbohydrate-binding proteins in cancer, and their ligands as therapeutic agents. *Trends Mol Med.* 8: 187-92.
- [26] Taniguchi N, Korekane H (2011) Branched N-glycans and their implications for cell adhesion, signaling and clinical applications for cancer biomarkers and in therapeutics. *BMB Rep.* 44: 772-81.
- [27] Cooper HS, Haesler WE Jr (1978) Blood group substances as tumor antigens in the distal colon. *Am J Clin Pathol.* 69: 594-8.
- [28] Madjd Z, Parsons T, Watson NF, Spendlove I, Ellis I, Durrant LG (2005) High expression of Lewis y/b antigens is associated with decreased survival in lymph node negative breast carcinomas. *Breast Cancer Res.* 7: 80-7.
- [29] St Hill CA, Farooqui M, Mitcheltree G, Gulbahce HE, Jessurun J, Cao Q, Walcheck B (2009) The high affinity selectin glycan ligand C2-O-sLex and mRNA transcripts of the core 2 beta-1,6-N-acetylglucosaminyltransferase (C2GnT1) gene are highly expressed in human colorectal adenocarcinomas. *BMC Cancer.* 9: 79.
- [30] Sobral AP, Rego MJ, Cavalcanti CL, Carvalho LB Jr, Beltrão EI (2010) ConA and UEA-I lectin histochemistry of parotid gland mucoepidermoid carcinoma. *J Oral Sci.* 52: 49-54.
- [31] de Lima AL, Cavalcanti CC, Silva MC, Paiva PM, Coelho LC, Beltrão EI, dos S Correia MT (2010) Histochemical evaluation of human prostatic tissues with *Cratylia mollis* seed lectin. *J Biomed Biotechnol.* 2010: 179817
- [32] Beltrão EI, Medeiros PL, Rodrigues OG, Figueredo-Silva J, Valença MM, Coelho LC, Carvalho LB Jr (2003) *Parkia pendula* lectin as histochemistry marker for meningothelial tumour. *Eur J Histochem.* 47: 139-42.

- [33] Narimatsu H, Sawaki H, Kuno A, Kaji H, Ito H, Ikehara Y (2010) A strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. *FEBS J.* 277: 95-105.
- [34] Rüegg C (2006) Leukocytes, inflammation, and angiogenesis in cancer: fatal attractions. *J Leukoc Biol.* 80: 682–684.
- [35] Ghobrial IM, Witzig TE, Adjei AA (2005) Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin.* 55: 178-94.
- [36] Michael OH (2000) The biochemistry of apoptosis. *Nature.* 407: 770–7.
- [37] Li CY, Xu HL, Liu B, Bao JK (2010) Concanavalin A, from an old protein to novel candidate anti-neoplastic drug. *Curr Mol Pharmacol.* 3: 123-8.
- [38] Oliveira C, Nicolau A, Teixeira JA, Domingues L (2011) Cytotoxic effects of native and recombinant frutalin, a plant galactose-binding lectin, on HeLa cervical cancer cells. *J Biomed Biotechnol.* 2011: 568932.
- [39] Huang LH, Yan QJ, Kopparapu NK, Jiang ZQ, Sun Y (2011) *Astragalus membranaceus* lectin (AML) induces caspase-dependent apoptosis in human leukemia cells. *Cell Prolif.* 45:15-21.
- [40] Lavrik IN, Golks A, Krammer PH (2005) Caspases pharmacological manipulation. *J Clin Invest.* 115: 2665–72.
- [41] Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE (2007) Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 β generation. *Clin Exp Immunol.* 147: 227-35.
- [42] Margetic S (2012) Inflammation and haemostasis. *Biochem Med (Zagreb).* 22: 49-62.
- [43] Hurwitz ZM, Ignatz R, Lalikos JF, Galili U (2012) Accelerated porcine wound healing after treatment with α -gal nanoparticles. *Plast Reconstr Surg.* 129: 242e-251e.
- [44] Di Lella S, Sundblad V, Cerliani JP, Guardia CM, Estrin DA, Vasta GR, Rabinovich GA (2011) When galectins recognize glycans: from biochemistry to physiology and back again. *Biochemistry.* 50: 7842-57.
- [45] Rabinovich GA, Toscano M, Jackson DA, Vasta G (2007) Functions of cell surface galectin-glycoprotein lattices. *Curr Opin Struct Biol.* 17: 513–520.
- [46] Rabinovich GA, Ilarregui JM (2009) Conveying glycan information into T-cell homeostatic programs: A challenging role for galectin-1 in inflammatory and tumor microenvironments. *Immunol Rev.* 230: 144–159.
- [47] Rabinovich GA, Toscano M (2009) Turning “sweet” on immunity: Galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol.* 9: 338–352.
- [48] Rodríguez RM, López-Vázquez A, López-Larrea C (2012) Immune systems evolution. *Adv Exp Med Biol.* 739: 237-51.
- [49] Fiocchi C (2011) Early versus late immune mediated inflammatory diseases. *Acta Gastroenterol Belg.* 74: 548-52.
- [50] Lee JY, Kim JY, Lee YG, Byeon SE, Kim BH, Rhee MH, Lee A, Kwon M, Hong S, Cho JY (2007) *In vitro* immunoregulatory effects of Korean mistletoe lectin on functional activation of monocytic and macrophage-like cells. *Biol Pharm Bull.* 30: 2043-51.
- [51] de Oliveira Silva F, das Neves Santos P, de Melo CM, Teixeira EH, de Sousa Cavada B, Arruda FV, Cajazeiras JB, Almeida AC, Pereira VA, Porto AL (2011)

- Immunostimulatory activity of ConBr: a focus on splenocyte proliferation and proliferative cytokine secretion. *Cell Tissue Res.* 346: 237-44.
- [52] de Melo CM, de Castro MC, de Oliveira AP, Gomes FO, Pereira VR, Correia MT, Coelho LC, Paiva PM (2010) Immunomodulatory response of Cramoll 1,4 lectin on experimental lymphocytes. *Phytother Res.* 24: 1631-6.
- [53] Brustein VP, Souza-Araújo FV, Vaz AF, Araújo RV, Paiva PM, Coelho LC, Carneiro-Leão AM, Teixeira JA, Carneiro-da-Cunha MG, Correia MT (2012) A novel antimicrobial lectin from *Eugenia malaccensis* that stimulates cutaneous healing in mice model. *Inflammopharmacology.* 20.
- [54] Neto LG, Pinto Lda S, Bastos RM, Evaristo FF, Vasconcelos MA, Carneiro VA, Arruda FV, Porto AL, Leal RB, Júnior VA, Cavada BS, Teixeira EH (2011) Effect of the lectin of *Bauhinia variegata* and its recombinant isoform on surgically induced skin wounds in a murine model. *Molecules.* 16: 9298-315.
- [55] Li B, Wang JH (2011) Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *J Tissue Viability.* 20: 108-20.
- [56] Boyd WC, Almodovar LR, Boyd LG (1966) Agglutinins in marine algae for human erythrocytes. *Transfusion* 6: 82-83.
- [57] Blunden G, Rogers DJ, Farnham WF (1975) Survey of British seaweeds for hemagglutinins. *Lloydia* 38: 162-168.
- [58] Kamiya H, Ogata K, Hori K (1982) Isolation and characterization of a new lectin in the red alga *Palmaria palmata* (L.) O. Kuntze. *Bot Marina.* 15: 537-540.
- [59] Fabregas J, Munoz A, Llovo J, Abalde J (1984) Agglutinins in marine red algae. *IRCS Medical Science* 12: 298-299.
- [60] Chiles TC, Bird KT (1989) A comparative study of animal erythrocyte agglutinins from marine algae. *Comp Biochem Physiol* 94: 107-111.
- [61] Ainouz IL, Sampaio AH (1991) Screening of Brazilian marine algae for hemagglutinins. *Bot Marina* 34: 211-214.
- [62] Hori K, Ikegami S, Miyazawa K, Ito K (1988) Mitogenic and antineoplastic isoagglutinins from red alga *Solieria robusta*. *Phytochemistry.* 27: 2063-2067.
- [63] Ainouz IL, Sampaio AH, Benevides NMB, Freitas ALP, Costa FHF, Carvalho MR, Pinheiro-Joventino F (1992) Agglutination of enzyme treated erythrocytes by Brazilian marine algal extracts. *Bot Marina* 35: 475-479.
- [64] Rogers DJ, Hori K (1993) Marine algal lectins: new developments. *Hydrobiologia*, 260/261: 589-593.
- [65] Hori K, Miyazawa K, Ito K (1990) Some common properties of lectins from marine algae. *Hydrobiologia.* 205: 561-566.
- [66] Ainouz IL, Sampaio AH, Freitas ALP, Benevides NMB, Mapurunga S (1995) Comparative study on hemagglutinins from the red marine algae *Bryothamnion triquetrum* and *B. Seaforthii*. *Rev Bras Fisiol Vegetal.* 7: 15-19.
- [67] Rogers DJ, Swain L, Carpenter BG, Critchley AT (1994) Binding of N-acetyl-D-galactosamine by lectins from species of green marine alga genus, *Codium*. *Clin Biochem.* 10: 162-165.

- [68] Sampaio AH, Rogers DJ, Barwell CJ (1998) A galactose specific lectin from the red marine alga *Ptilota filicina*. *Phytochemistry* 48: 765-769.
- [69] Sampaio AH, Rogers DJ, Barwell CJ, Saker-Sampaio S, Costa FHF, Ramos MV (1999) A new isolation and further characterization of the lectin from the red marine alga *Ptilota serrata*. *J Appl Phycol* 10: 539-546.
- [70] Sampaio AH, Rogers DJ, Barwell CJ, Saker-Sampaio S, Nascimento KS, Nagano CS, Farias WRL (2002) New affinity procedure for the isolation and further characterization of the blood group B specific lectin from the red marine alga *Ptilota plumosa*. *J Appl Phycol*. 14: 489-496.
- [71] Sharon N, Lis H (1990) Legume lectins – a large family of homologous proteins. *FASEB J*. 4: 3198-3208.
- [72] Rogers DJ, Fish B, Barwell CJ, Loveless RW (1988) Lectins from marine algae associated with photosynthetic accessory proteins. In: INTERNATIONAL LECTIN MEETING, Berlin/New York. Proceedings of the 9th Lectin Meeting. Berlin/New York: Walter de Gruyter. 6: 373-376.
- [73] Costa FHF, Sampaio AH, Neves SA, Rocha MLA, Benevides NMB, Freitas ALP (1999) Purification and partial characterization of a lectin from the red marine alga *Amansia multifida*. *Physiol Mol Biol Plants*. 5:53-61.
- [74] Kamiya H, Shiomi K, Shimizu Y (1980) Marine biopolymers with cell specificity III agglutinins in the red alga *Cystoclonium purpureum*: isolation and characterization. *J Natur Products*. 43: 136-139.
- [75] Shiomi K, Yamanaka H, Kikuchi T (1981) Purification and physicochemical properties of a hemagglutinin (GVA-1) in the red alga *Gracilaria verrucosa*. *Bull Jap Soc Sci Fisheries*. 47: 1079-1084.
- [76] Kamiya H, Ogata K, Hori K (1982) Isolation and characterization of a: new agglutinin in the red alga *Palmaria palmata* (L.) O. Kuntze. *Bot Marina*. 25: 537-540.
- [77] Benevides NMB, Leite AM, Freitas ALP (1996) Atividade hemaglutinante na alga vermelha *Solieria filiformis*. *R Bras Fisiol Vegetal* 8: 117-122.
- [78] Benevides NMB, Holanda ML, Melo FR, Freitas ALP, Sampaio AH (1998) Purification and partial characterization of the lectin from the red marine alga *Enantiocladia duperreyi* (C. Agardh) Faalkenberg. *Bot Marina* 41: 521-525.
- [79] Nagano CS, Moreno FB, Bloch Jr C, Prates MV, Calvete JJ, Saker-Sampaio S, Farias WR, Tavares TD, Nascimento KS, Grangeiro TB, Cavada BS, Sampaio AH (2002) Purification and characterization of a new lectin from the red marine alga *Hypnea musciformis*. *Protein Pept Lett*. 9: 159-66.
- [80] Leite YFMM, Silva LMCM, Amorim RCN, Freire EA, Jorge DMM, Grangeiro TB, Benevides NMB (2005) Purification of a lectin from the marine red alga *Gracilaria ornata* and its effect on the development of the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Bioch et Bioph Acta*. 1724: 137-145.
- [81] Nascimento KS, Nagano CS, Nunes EV, Rodrigues RF, Goersch GV, Cavada BS, Calvete JJ, Saker-Sampaio S, Farias WRL, Sampaio AH (2006) Isolation and characterization of a new agglutinin from the red marine alga *Hypnea cervicornis* J. Agardh. *Biochem Cel Biol*. 84: 49-54.

- [82] Souza BWS, Andrade FK, Teixeira DIA, Mansilla A, Freitas ALP (2010) Haemagglutinin of the antarctic seaweed *Georgiella confluens* (Reinsch) Kylin: isolation and partial characterization. *Polar Biol.* 1: 1-8.
- [83] Rogers DJ, Loveless RW, Balding P (1986) Isolation and characterization of the lectins from sub-species of *Codium fragile*. In. INTERNATIONAL LECTIN MEETING, Berlin/New York. Proceedings of the 7th Lectin Meeting. Berlin/New York: Walter de Gruyter. 5: 155-160.
- [84] Fabregas J, Muñoz A, Llovo J, Carracedo A (1988) Purification and partial purification of tomentine. An N-acetylglucosamine-specific lectin from green alga *Codium tomentosum* (huds) Stackh. *J Exp Mar Biol Ecology.* 124: 21-30.
- [85] Rogers DJ, Flangu H (1991) Lectins from *Codium* species. *Br Phycol J.* 26: 95-96.
- [86] Sampaio AH, Rogers DJ, Barwell CJ (1998) Isolation and characterization of the lectin from the green marine alga *Ulva lactuca*. *Bot Marina* 41: 765-769.
- [87] Benevides NMB, Holanda ML, Melo FR, Pereira MG, Monteiro ACO, Freitas ALP (2001) Purification and partial characterization of the lectin from the marine green alga *Caulerpa cupressoides* (Vahl) C. Agardh. *Bot Marina* 44: 17-22.
- [88] Ambrosio A, Sanz L, Sanchez EI, Wolfenstein-Todel C, Calvete JJ (2003) Isolation of two novel mannan- and L-fucose-binding lectins from the green alga *Enteromorpha prolifera*: biochemical characterization of EPL-2. *Archives of Biochemistry and Biophysics* 415: 245-250.
- [89] Wang S, Zhong FD, Zhang YJ, Wu ZJ, Lin QY, Xie LH (2004) Molecular characterization of a new lectin from the marine alga *Ulva pertusa*. *Acta Biochim Biophys Sin.* 36: 111-117.
- [90] Kim GH, Klochkova TA (2005) Purification and Characterization of a Lectin, Bryohealin, Involved in the Protoplast Formation of a Marine Green Alga *Bryopsis Plumosa* (Chlorophyta). *J Phycol.* 42: 86-95.
- [91] Han JW, Jung MG, Kim MJ, Yoon KS, Lee KP, Kim GH (2010) Purification and characterization of a D-mannose specific lectin from the green marine alga, *Bryopsis plumose*. *Phycol Res.* 58: 143-150.
- [92] Han JW, Yoon KS; Klochkova TA, Hwang M-S, Kim GH (2011) Purification and characterization of a lectin, BPL-3, from the marine green alga *Bryopsis plumosa*. *J Appl Phycol.* 23: 745-753.
- [93] Niu J, Wang G, Lü F, Zhou B, Peng G (2009) Characterization of a new lectin involved in the protoplast regeneration of *Bryopsis hypnoides*. *Chin J Oceanol Limnol.* 27: 502-512.
- [94] Loomis WD (1974) Overcoming problems of phenolics and quinines in the isolation of plant enzymes and organelles. *Methods Enzymol.* 16: 528-544.
- [95] Blunden G, Roger DJ, Loveless RW, Patel A.V (1986) Haemagglutinins in marine algae: Lectins or Phenols? In: "Lectins: Biology, Biochemistry, Clin Biochem. 5: 139-145.
- [96] Calvete JJ, Costa FHF, Saker-Sampaio S, Moreno-Murciano MP, Nagano CS, Cavada BS, Grangeiro TB, Ramos MV, Bloch Jr C, Silveira SB, Freitas BT, Sampaio AH (2000) The amino acid sequence of the agglutinin isolated from the red marine alga *Bryothamnion triquetrum* defines a novel lectin structure. *Cell Mol Lif Sci* 57: 343-350.

- [97] Hori K, Matsubara K, Miyazawa K (2000) Primary structures of two hemagglutinins from the red alga *Hypnea japonica*. *Bioch Biophys Acta*. 1474: 226-236.
- [98] Medina-Ramirez G, Gibbs RV, Calvete JJ (2006) Micro-heterogeneity and molecular assembly of the haemagglutinins from the red algae *Bryothamnion seaforthii* and *B. triquetrum* from the Caribbean Sea. *Eur J Phycol*. 42: 105-112.
- [99] Nagano CS, Debray H, Nascimento KS, Pinto VPT, Cavada BS, Saker-Sampaio S, Farias WRL, Sampaio AH, Calvete JJ (2005) HCA and HML isolated from the red marine algae *Hypnea cervicornis* and *Hypnea musciformis* define a novel lectin family. *Protein Sci*. 14: 2167-2176.
- [100] Kawabuko A, Makino H, Ohnishi J, Hirohara H, Hori K (1999) Occurrence of highly yielded lectins homologous within the genus *Eucheuma*. *J Appl Phycol*. 11: 149-156.
- [101] Hori K, Sato Y, Ito K, Fujiwara Y, Iwamoto Y, Makino H, Kawakubo A (2007) Strict specificity for high-mannose type N-glycans and primary structure of a red alga *Eucheuma serra* lectin. *Glycobiology*, 17: 479-9.
- [102] Mori T, O'Keefe BR, Sowder II RC, Bringans S, Gardella R, Berg S, Cochran P, Turpin JA, Buckheit Jr RW, MacMahon JB, Boyd MR (2005) Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga *Griffithsia* sp. *J Biol Chem*, 280: 9345-9353.
- [103] Ishihara K, Arai S, Shimada S (2009) cDNA cloning of a lectin-like gene preferentially expressed in freshwater from macroalga *Ulva limnetica* (ulcales, Chlorophyta). *Phycol Res* 57: 104-110.
- [104] Yoon KS, Lee KP, Klochkova TA, Kim GH (2008) Molecular characterization of the lectin, bryohealin, involved in protoplast regeneration of the marine alga *Bryopsis plumosa* (chlorophyta). *J Phycol* 44: 103-112.
- [105] Sato Y, Hirayama M, Morimoto K, Yamamoto N, Okuyama S, Hori K (2011) High Mannose-binding Lectin with preference for the cluster of α 1-2 Mannose from green Alga *Boodlea coacta* is potent inhibitor of HIV-1 and Influenza Viruses. *J Biol Chem*. 286: 19446-19458.
- [106] Sugahara T, Ohama Y, Fukuda A, Hayashi M, Kawakubo A, Kato K (2001) The cytotoxic effect of *Eucheuma serra* agglutinin (ESA) on cancer cells and its application to molecular probe for drug delivery system using lipid vesicles. *Cytotechnology*. 36: 93-99.
- [107] Fukuda Y, Sugahara T, Ueno M, Fukuta Y, Ochi Y, Akiyama K, Miyazaki T, Masuda S, Kawakubo A, Kato K (2006) The anti-tumor effect of *Eucheuma serra* agglutinin on colon cancer cells *in vitro* and *in vivo*. *Anticancer Drugs*. 17: 943-7.
- [108] Omokawa Y, Miyazaki T, Walde P, Akiyama K, Sugahara T, Masuda S, Inada A, Ohnishi Y, Saeki T, Kato K (2010) In vitro and in vivo anti-tumor effects of novel Span 80 vesicles containing immobilized *Eucheuma serra* agglutinin. *Int J Pharm*. 15: 157-167.
- [109] Pinto VP, Debray H, Dus D, Teixeira EH, de Oliveira TM, Carneiro VA, Teixeira AH, Filho GC, Nagano CS, Nascimento KS, Sampaio AH, Cavada BS (2009) Lectins from the Red Marine Algal Species *Bryothamnion seaforthii* and *Bryothamnion triquetrum* as Tools to Differentiate Human Colon Carcinoma Cells. *Adv Pharmacol Sci*. 2009: 862162.

- [110] Bitencourt FS, Figueiredo JG, Mota MR, Bezerra CC, Silvestre PP, Vale MR, Nascimento KS, Sampaio AH, Nagano CS, Saker-Sampaio S, Farias WR, Cavada BS, Assreuy AM, de Alencar NM (2008) Antinociceptive and anti-inflammatory effects of a mucin-binding agglutinin isolated from the red marine alga *Hypnea cervicornis*. *Naunyn Schmiedebergs Arch Pharmacol.* 377: 139-48.
- [111] Silva LM, Lima V, Holanda ML, Pinheiro PG, Rodrigues JA, Lima ME, Benevides NM (2010) Antinociceptive and anti-inflammatory activities of lectin from marine red alga *Pterocladia capillacea*. *Biol Pharm Bull.* 33: 830-5.
- [112] Vanderlei ES, Patoilo KK, Lima NA, Lima AP, Rodrigues JA, Silva LM, Lima ME, Lima V, Benevides NM (2010) Antinociceptive and anti-inflammatory activities of lectin from the marine green alga *Caulerpa cupressoides*. *Int Immunopharmacol.* 10: 1113-8.
- [113] Hori K, Matsuda H, Miyazawa K, Ito K (1987) A mitogenic agglutinin from the red alga *Carpopeltis flabellate*. *Phytochemistry.* 26: 1335-1338.
- [114] Liao WR, Lin JY, Shieh WY, Jeng WL, Huang R (2003) Antibiotic activity of lectins from marine algae against marine vibrios. *J Ind Microbiol Biotechnol.* 30: 433-439.
- [115] Teixeira EH, Napimoga MH, Carneiro VA, de Oliveira TM, Nascimento KS, Nagano CS, Souza JB, Havt A, Pinto VP, Gonçalves RB, Farias WR, Saker-Sampaio S, Sampaio AH, Cavada BS (2007) *In vitro* inhibition of oral streptococci binding to the acquired pellicle by algal lectins. *J Appl Microbiol.* 103: 1001-6.
- [116] Neves SA, Dias-Baruff M, Freitas AL, Roque-Barreira MC (2001) Neutrophil migration induced in vivo and in vitro by marine algal lectins. *Inflamm Res.* 50: 486-90.
- [117] Sato Y, Morimoto K, Hirayama M, Hori K (2011) High mannose-specific lectin (KAA-2) from the red alga *Kappaphycus alvarezii* potentially inhibits influenza virus infection in a strain-independent manner. *Biochem Biophys Res Commun.* 405: 291-6.
- [118] Mori T, O'Keefe BR, Sowder RC 2nd, Bringans S, Gardella R, Berg S, Cochran P, Turpin JA, Buckheit RW Jr, McMahon JB, Boyd MR. Isolation and characterization of griffithsin, a novel HIV-inactivating protein, from the red alga *Griffithsia sp.* *J Biol Chem.* 280: 9345-53.
- [119] Emau P, Tian B, O'keefe BR, Mori T, McMahon JB, Palmer KE, Jiang Y, Bekele G, Tsai CC (2007) Griffithsin, a potent HIV entry inhibitor, is an excellent candidate for anti-HIV microbicide. *J Med Primatol.* 36: 244-53.
- [120] O'Keefe BR, Giomarelli B, Barnard DL, Shenoy SR, Chan PK, McMahon JB, Palmer KE, Barnett BW, Meyerholz DK, Wohlford-Lenane CL, McCray PB Jr (2010) Broad-spectrum *in vitro* activity and *in vivo* efficacy of the antiviral protein griffithsin against emerging viruses of the family *Coronaviridae*. *J Virol.* 84: 2511-21.
- [121] Abee T, Kovács AT, Kuipers OP, Van Der Veen S (2010) Biofilm formation and dispersal in Gram-positive bacteria. *Curr Opin Biotechnol.* 22: 1-8.
- [122] Merritt J, Anderson MH, Park NH, Shi W (2001) Bacterial biofilm and dentistry. *J Calif Dent Assoc.* 29: 355-60.
- [123] Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev.* 15: 167-193.

- [124] Onurdağ FK, Ozkan S, Ozgen S, Olmuş H, Abbasoğlu U (2010) *Candida albicans* and *Pseudomonas aeruginosa* adhesion on soft contact lenses. Graefes Arch Clin Exp Ophthalmol. Dez. 2010.
- [125] Prosser BT, Taylor PA, Cix PA, Cluland R (1987) Method of evaluating effects of antibiotics on bacterial biofilms. Antimicrob. Agents Chemother. 31: 1502-1506.
- [126] Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Ann. Rev. Microbiol. 49: 711-745.
- [127] Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9(1): 34-39.
- [128] Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: A common cause of persistent infections. Scien. 284: 1318-1322.
- [129] Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G (2003) The application of biofilm science to the study and control of chronic bacterial infections. J. Clin. Invest. 112: 146-1477.
- [130] Fux CA, Stoodley P, Hall-Stoodley, Costerton JW (2003) Bacterial biofilms: a diagnostic and therapeutic challenge. Exp Rev Anti-infect Ther. 1(4): 667-683.
- [131] Alhede M, Bjarnsholt T, Jensen PO (2009) *Pseudomonas aeruginosa* recognizes and responds aggressively to the presence of polymorphonuclear leukocytes. Microbiol. 155: 3500-3508.
- [132] Van Gennip M, Christensen LD, Alhede M (2009) Inactivation of the *rhlA* gene in *Pseudomonas aeruginosa* prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes. APMIS. 117: 537-546.
- [133] Busscher HJ, Bos R, van der Mei HC (1995) Initial microbial adhesion is a determinant for the strength of biofilm adhesion. FEMS Microbiol. Lett. 128: 229-234.
- [134] Wu C, Mishra A, Reardon ME, Huang IH, Counts SC, Das A, Ton-That H (2012) Structural determinants of *Actinomyces sortase* SrtC2 required for membrane localization and assembly of type 2 fimbriae for interbacterial coaggregation and oral biofilm formation. J Bacteriol. Mar 23.
- [135] Periasamy S, Kolenbrander PE (2010) Central role of the early colonizer *Veillonella sp.* in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. J Bacteriol. 192(12): 2965-72.
- [136] Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol. 56: 187-209.
- [137] Heydorn A, Ersboll BK, Hentzer M, Parsek MR, Givskov M, Molin S (2000) Experimental reproducibility in flow-chamber biofilms. Microbiol. 146: 2395-2407.
- [138] Xiao J, Klein MI, Falsetta ML, Lu B, Delahunty CM, Yates JR 3rd, Heydorn A, Koo H (2012) The Exopolysaccharide matrix modulates the interaction between 3D architecture and virulence of a mixed-species oral biofilm. PLoS Pathog. 8(4): e1002623.
- [139] López D, Vlamakis H, Kolter R (2010) Biofilms. Cold Spr Harb Perspect Biol. 2(7): a000398.
- [140] Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells". J Bacteriol. 189(22): 7945-7.
- [141] Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol. 8(9): 623-33.

- [142] Fronzes R, Remaut H, Waksman G (2008) Architectures and biogenesis of non-flagellar protein appendages in Gram-negative bacteria. *EMBO J.* 27: 2271-80.
- [143] Dorkhan M, Chávez de Paz LE, Skepö M, Svensäter G, Davies J (2012) Effects of saliva or serum coating on adherence of *Streptococcus oralis* strains to titanium. *Microbiol.* 158(2): 390-7.
- [144] Mukherjee J, Karunakaran E, Biggs CA (2012) Using a multi-faceted approach to determine the changes in bacterial cell surface properties influenced by a biofilm lifestyle. *Biofouling.* 28(1): 1-14.
- [145] Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. *Adv. Dent. Res.* 8: 263-271.
- [146] Nyvad B (1993) Microbial colonization of human tooth surfaces. *APMIS Suppl.* 32: 1-45.
- [147] Thylstrup A, Fejerskov O (1995) *Cariologia clínica* 2 ed. São Paulo: Santos, Cap. 3, p. 45-49.
- [148] Ruhl S, Sandberg AL, Cisar JO (2004) Salivary receptors for the proline-rich protein-binding and lectin-like adhesins of oral actinomyces and streptococci. *J Dent Res.* 83(6): 505-10.
- [149] Jenkinson HF, Lamont RJ (1997) Streptococcal adhesion and colonization. *Crit Rev Oral Biol Med.* 8(2): 175-200.
- [150] Korea GK, Ghigo JM, Beloin C (2011) The sweet connection: Solving the riddle of multiple sugar-binding fimbrial adhesins in *Escherichia coli*: Multiple *E. coli* fimbriae form a versatile arsenal of sugar-binding lectins potentially involved in surface-colonisation and tissue tropism. *Bioessays* 33: 300-311.
- [151] Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer Jr RJ (2002) Communication among oral bacteria. *Microbiol Mol Biol Rev.* 66: 486-505.
- [152] Ferreira CL, Grzeńkowiak L, Collado MC, Salminen S (2011) In vitro evaluation of *Lactobacillus gasseri* strains of infant origin on adhesion and aggregation of specific pathogens. *J Food Prot.* 74(9): 1482-7.
- [153] Okuda T, Okuda K, Kokubu E, Kawana T, Saito A, Ishihara K (2012) Synergistic effect on biofilm formation between *Fusobacterium nucleatum* and *Capnocytophaga ochracea*. *Anaerobe.* 18(1): 157-61.
- [154] Handley PS, Rickard AH, High NJ, Leach SA (2001) Coaggregation - is it a universal phenomenon? In: *Biofilm community interactions: Chance or Necessity* (Gilbert, P., Allison, D., Verran, J., Brading, M. and Walker, J., Eds.), pp. 1-10.
- [155] Rodrigues DF, Elimelech M (2009) Role of type 1 fimbriae and mannose in the development of *Escherichia coli* K12 biofilm: from initial cell adhesion to biofilm formation. *Biofoul.* 25(5): 401-11.
- [156] Holmes AR, Gopal PK, Jenkinson HF (1995) Adherence of *Candida albicans* to a cell surface polysaccharide receptor on *Streptococcus gordonii*. *Infect Immun.* 63(5): 1827-34.
- [157] Rosen G, Sela MN (2006) Coaggregation of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* PK 1594 is mediated by capsular polysaccharide and lipopolysaccharide. *FEMS Microbiol Lett.* 256(2): 304-10.

- [158] Ryu JH, Beuchat LR (2005) Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. *Appl Environ Microbiol.* 71(1): 247-54.
- [159] Simões LC, Lemos M, Pereira AM, Abreu AC, Saavedra MJ, Simões M (2011) Persister cells in a biofilm treated with a biocide. *Biofoul.* 27(4): 403-11.
- [160] Becer CR (2012) The glycopolymer code: Synthesis of glycopolymers and multivalent carbohydrate-lectin interactions. *Macromol Rapid Commun.* Apr 16.
- [161] Kurz K, Garimorth K, Joannidis M, Fuchs D, Petzer A, Weiss G (2012) Altered immune responses during septicaemia in patients suffering from haematological malignancies. *Int J Immunopathol Pharmacol.* 25(1): 147-56.
- [162] Strathmann, M, Wingender J, Flemming H (2002) Application of fluorescently labelled lectins for the visualization and biochemical characterization of polysaccharides in biofilms of *Pseudomonas aeruginosa*. *J. Microbiol. Methods* 50: 237-248.
- [163] Wawrzynczyk J, Szewczyk E, Norrlöw O, Dey E (2007) Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge. Characterization of the extracted polysaccharides/glycoconjugates by a panel of lectins. *J. Biotechnol.* 130: 274-281.
- [164] Cavalcante TT, Rocha BAM, Carneiro VA, Arruda FVS, AS FN, Sá NC, Nascimento KS, Cavada BS, Teixeira EH (2011) Effect of lectins from Diocleinae subtribe against oral *Streptococci*. *Molecul.* 16(5): 3530-43.
- [165] Sanford BA; De Feijter AW, Wade MH, Thomas VL (1996) A dual fluorescence technique for visualization of *Staphylococcus epidermidis* biofilm using scanning confocal laser microscopy. *J Ind Microbiol.* 16(1): 48-56.
- [166] Thomas VL, Sanford BA, Moreno R, Ramsay MA (1997) Enzyme-linked lectin sorbent assay measures N-acetyl-D-glucosamine in matrix of biofilm produced by *Staphylococcus epidermidis*. *Curr Microbiol.* 35(4): 249-54.
- [167] McCoy Jr JP, Varani J, Goldstein IJ (1984) Enzyme-linked lectin assay (ELLA): Detection of carbohydrate groups on the surface of unfixed cells. *Exper Cell Res.* 1(51): 96-103.
- [168] Leriche V, Sibille P, Carpentier B (2000) Use of an enzyme-linked lectin sorbent assay to monitor the shift in polysaccharide composition in bacterial biofilms. *Appl Environ Microbiol.* 66(5): 1851-6.
- [169] Zippel B, Neu TR (2011) Characterization of glycoconjugates of extracellular polymeric substances in tufa-associated biofilms by using fluorescence lectin-binding analysis. *Appl Environm Microbiol.* 77: 505-516.
- [170] Rebiere-Huët J, Di Martino P, Hulen C. (2004) Inhibition of *Pseudomonas aeruginosa* adhesion to fibronectin by PA-IL and monosaccharides: involvement of a lectin-like process. *Can J Microbiol.* 50(5): 303-12.
- [171] Teixeira EH, Napimoga MH, Carneiro VA, de Oliveira TM, Cunha RM, Havt A, Martins JL, Pinto VP, Gonçalves RB, Cavada BS (2006) In vitro inhibition of *Streptococci* binding to enamel acquired pellicle by plant lectins. *J Appl Microbiol.* 101(1): 111-6.
- [172] Islam B, Khan SN, Naeem A, Sharma V, Khan AU (2009) Novel effect of plant lectins on the inhibition of *Streptococcus mutans* biofilm formation on saliva-coated surface. *J Appl Microbiol.* 106(5): 1682-9.