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Review

Plant lectins as alternative tools against bacterial biofilms

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Plant lectins has the ability to interfere in crucial biofilm formation aspects, playing a significant role in evaluation of patients at high and low risk of disease development. However, there is limited studies on the physiological role of lectins on bacteria living in biofilms like caries process. Thus, we aimed to provide a view of lectins biotechnological potential against bacterial biofilms development. Biofilm is a structured bacterial consortium that provides essential compounds for its survivor. This microorganism organization occurs naturally, since this arrangement increases its survival possibility. Bacterial biofilms are related to human health problems and are responsible for many infectious diseases, such as oral diseases, associated with inert surfaces, including medical devices for internal and external use. Thus, lectins are a large group of heterogeneous proteins that exhibit antibacterial activity, as well as ability to interfere with microbial biofilms formation process. The lectins ability to form complexes with microbial glycoconjugates has stimulated its application as probes to the whole cell, as well as its mutants and numerous cellular constituents and metabolites. Thus, the impact of bacterial resistance provided by biofilm formation on human health encourages researches aiming to understand biofilm mechanisms as well as strategies to eradicate or minimize these communities damages.

Key words: Lectins, biofilm, bacterial resistance.

INTRODUCTION

Plant lectins have been used for diagnosis and prevention of various diseases. This is justified by property set translated by the ability to recognize structural elements of organizational surface from pathogens (Cavalcante et al., 2011). However, when it comes to interaction with bacterial biofilms, this strategy has been incipiently exploited (Lopes et al., 2005).

Given this context, this study proposes the following question: what are the potential of biotechnological application of plant lectins in diagnostics and disease

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prevention?

Since lectins, as molecules, have the ability to bind glicoconjugates (crucial function for biofilm formation), these proteins may play a significant role in the evaluation of patients at high and low risk for biofilm associated disease development.

In dentistry, there are few studies related to the use of lectins in the diagnosis and prevention of diseases. Studies on this subject also failed to respond effectively, and the physiological role of lectins are against the caries process. Some authors suggest various proposals regarding to this question and present meaningful data, especially with regard to the actions of lectins of higher plants (Teixeira et al., 2007; Cavalcante et al., 2011; Klafke et al., 2013).

Thus, this study aims to give a view of the biotechnological potential of plant lectins as a tool to deal with bacterial biofilms as a source of bacterial resistance.

MICROBIAL BIOFILMS: CHARACTERISTICS, FORMATION AND FUNCTIONS

Biofilm characteristics

The microbial word observation using different microscopy techniques over the years, has provided accurate researches of the microorganisms arranged in communities sharing nutrients, metabolites, genetic elements and thus, being able to resist even in withstand environment, causing diseases that could be difficult to eliminate. Biofilms have a crucial impact on human health in different ways, since they can be formed in natural environments, medical devices and industrial equipment (Lopez et al., 2010).

The majority of microorganism naturally aggregates and produces a self-produced polysaccharide matrix called a biofilm (McDougald et al., 2012). These communities may be established in a wide variety of surfaces (Abee et al., 2011).

Besides the ability to produce extracellular polymers, cells in communities presents a reduced pattern of growth, as well as up or down regulation of specific genes. The organization of microorganisms in biofilms occurs naturally, since these communities arrangement increases the possibility of survival of these microscopic organisms. Synthesis of extracellular polymeric substances by microorganisms is accepted as a key mechanism to facilitate irreversible cell adhesion to inanimate surfaces in wet environments, thus promoting the development of a biofilm (Beech et al., 2005). The presence of the ‘‘matrix of extracellular polymeric substance’’, which contains polysaccharides, proteins and DNA, whose formation is a consequence of the metabolism of the microbial community is one specific characteristic of bacterial biofilm (Erriu et al., 2013).

These communities also display a particular profile, since they can host different species of microorganisms in an arrangement that allows cooperation instead of competition (Bordi and Bentzmann, 2011). A communication system between bacterial species is responsible for the development and integrity of the biofilm structure. These system synthetize pheromones that allow cell-to-cell communication which induce the biofilm-forming bacteria to react as one against external stress. This Quorum Sensing (QS), chemical communication between bacterial cells, is closely involved both in biofilm formation and in surface motility in pathogens, and whose activation is linked to molecules auto-inducers (AIs) (Aparna and Yadav, 2008; Karatuna and Yagci, 2010).

Furthermore, these microbial societies have their own rules and behavior, including altruism and cooperation, which benefits the group (Shapiro, 1998; Parsek and Greenberg, 2005). Some of these subpopulations can exhibit expertise that is orchestrated by chemical communications (Weigel et al., 2007) providing a singular way of interaction among species, inducing marked changes on symbiotic relationships between their components (Hansen et al., 2007).

Biofilm formation

The knowledge of the molecular basis involved in biofilm development has been updated by improvements in methods for genetic and genomic studies, as well as the development of laboratorial technology, that reveals the processes involved on development, physiology and behavior of microorganisms in this new environment condition. For example, a plethora of systems allows the bacterial identification, appropriated surface anchoring and cell adhesion to form multicellular communities (Bordi and Bentzmann, 2011). Bacterial growth in pure media conditions has been the main approach to perform microbiological culture, from Pasteur’s studies to the present day. These experiments have been used to provide knowledge and understanding of prokaryotic genetic and metabolism, further facilitating pathogens from a variety of diseases isolation and identification of diseases (Costerton et al., 1987).

The term biofilm was introduced with evidence that bacterial behavior associated with surfaces could not be predicted by observations performed in microorganisms cultured in suspension, in their planktonic form. This is a term that describes sessile microbial populations introduced through surveys of biofilms (Jakubovics and Kolenbrander, 2010).

Biofilm formation may be considered a bacterial community protective mechanism against external injury, thus, it seems reasonable that extracellular signals regulate the activation of specific metabolic patterns that trigger its stability. Such signaling may arise from various

external sources, and can be produced and secreted by the bacterial community itself, where molecules named self-inducers accumulate in the extracellular medium with concentrations correlated with population density (Lopez et al., 2010), and may trigger signaling cascades that lead to responses in multicellular bacterial population, when in high concentrations. This mechanism of cell-cell communication (called *quorum sensing*) controls a large amount of processes including those related to biofilm formation (Camilli and Bassler, 2006). Furthermore, each bacterial species has its own apparatus to accomplish adhesion, and contains a different number of antagonic or sinergistic molecules which are cell specific and can be released depending on the situation (Hagan et al., 2010).

The process of biofilm formation (Figure 1) has been extensively described (Costerton et al., 1995; Habash and Reid, 1999; Donlan and Costerton, 2002), and involves few steps: an initial reversible connection of plaktonic cells to a surface followed by a maturation phase. This initial binding involves attractive and repulsive forces between cells and surface, which include electrostatic and hydrophobic interactions, van der Waals and hydrodynamic forces at appropriated temperature (Agarwal et al., 2010). After this surface binding, bacterial cells grow and divide to form dense clusters of cells that characterize the biofilm. This phase is associated with the polysaccharide production by bacterial cells, and become irreversibly adhered to the substrate. Temporally, these microcolonies develop into a mature biofilm, acquiring a typical architecture with projections separated by channels filled with fluid. The final stage (dispersion phase) involves the shutdown of cells or groups of cells from mature biofilm, being considered an essential step in the bacterial spread (Santos et al., 2008; Batoni et al., 2011).

As far as the cell surface hydrophobicity and the presence of fimbriae and flagella is concerned, exopolissacaride production is one of the main factors that influence the rate and degree of microbial cell adhesion on different surfaces and protects against environmental stress and dehydration (Vu et al., 2009). The extracellular material is mostly produced by the biofilm cells forming. It consists of different types of biomolecules, designated as extracellular polymeric substances (EPS), that forms the scaffold for the three-dimensional architecture of the biofilm and is linked to cell adhesion to surfaces and for cohesion in the biofilm (Flemming and Wingender, 2010). Thus, the EPS production has been the subject of several studies to impair formation and maturation of these microbial communities (Murray et al., 2009; Nagorska et al., 2010).

Correlations of biomedical interest

Bacterial biofilms are related to human health problems responsible for many infectious diseases associated with inert surfaces, including medical devices for internal and external use. They could also be formed in water pipes in hospitals, leading to infections after admission (Bordi and Bentzmann, 2011). The relevance of biofilm formation on medical devices, such as catheters or implants, can result in chronic infections difficult to treat (Hall-Stoodley et al., 2004; Donlan, 2008; Hatt and Rather, 2008). Chronic infections with biofilms involvement include periodontitis, cystic fibrosis pneumonia, and others infections associated with indwelling devices such as catheters, heart valves and prostheses (Stewart, 2002).

Confocal microscopy evidenced that live mature biofilms are not single structured layers in a microbial cell surface, but a heterogeneous entities in time and space, constantly changing due to external and internal processes (Donlan and Costerton, 2002). A biofilm may be composed by bacterial or fungal species, or several species of bacterial, fungal and even algae and protozoa (Batoni et al., 2011). Chemical compounds formed by only a micro-organism may also be present in some infectious pathologies, such as in biofilms formed in heart valves infective endocarditis consisting of *Staphylococcus epidermidis* (Butany et al., 2002). Furthermore, infections have been associated with the formation of biofilms on surfaces such as human tooth, skin and urinary tract (Hatt and Rather, 2008). This community organization provides microbial resistance to various antimicrobial, protection from protozoa and host defenses (Matz and Kjelleberg, 2005; Anderson and O'toole, 2008).

It has been recently reported that 95-99% of microorganisms occurs naturally in biofilms arrangements (Nikolaev and Plakunov, 2007). These microbial communities protect their residents not only from oxygen but also from other environmental factors (Paerl and Pinckney, 1996). Bacterial growing in biofilms causes chronic infections (Costerton et al., 2003) which are characterized by persistent inflammation and tissue damage (Bjarnsholt et al., 2009). Chronic infections, including foreign body infections, are 1) persistent despite antibiotic therapy and host innate and adaptive immune system and inflammatory response and 2) in contrast to colonization, is characterized by pathological immune response and disease persistence (Hoiby et al., 2010).

Mechanisms of antimicrobial resistance

Microorganisms belonging to these microbial communities exhibit particular properties, such as tolerance and resistance to different drugs, opsonization and phagocytosis, allowing them to survive in harsh environments and resist t selective pressures (Weitao, 2009). It seems that host immunity is ineffective in "clean" these microcommunities, since evidence shows the inability of phagocytic cells to act (Leid et al., 2002) or

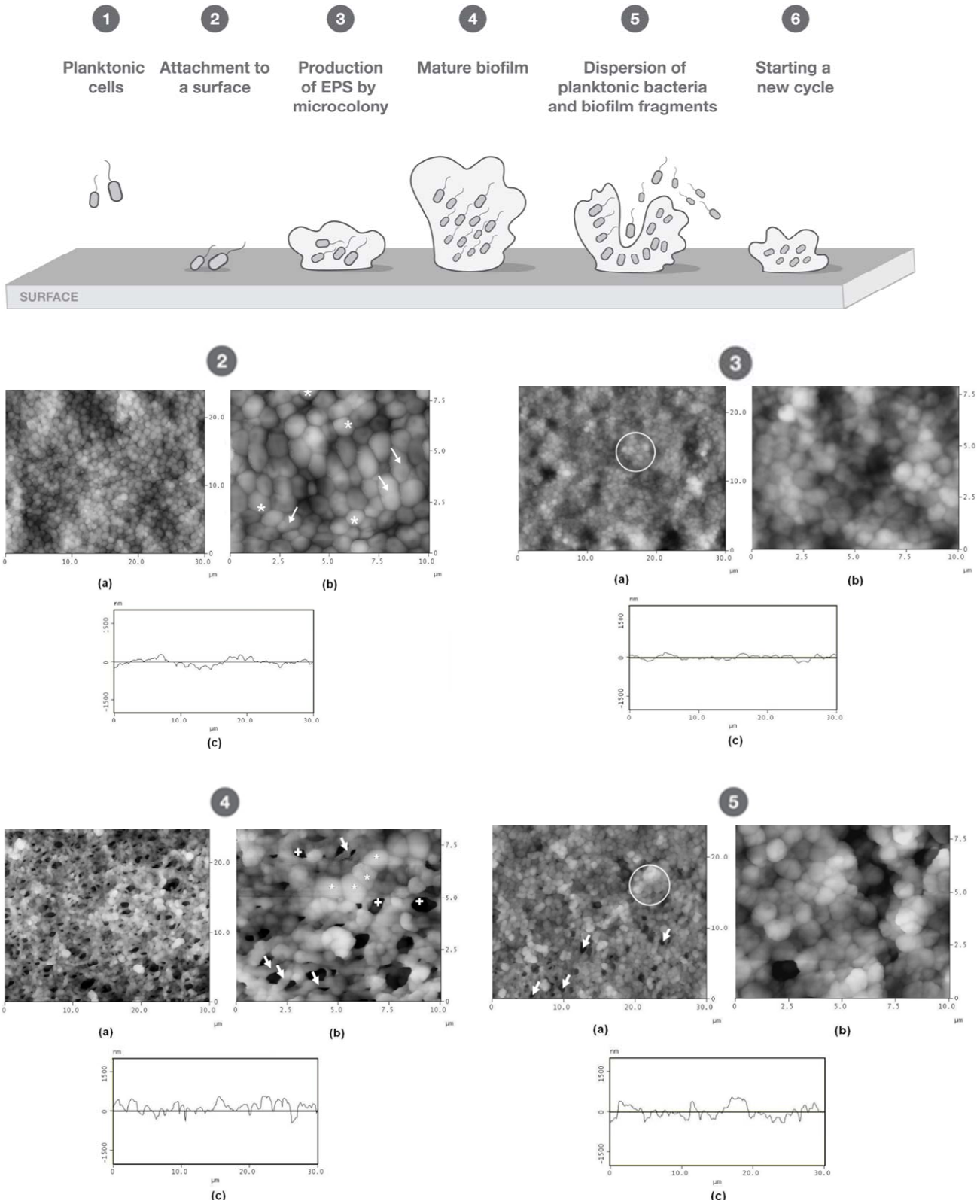

Figure 1. 2a, 3a, 4a, and 5a: Atomic Force Microscopy (AFM) 2D images with sacanning of 30x30 µm of cellulose nitrate membrane (CNM). 2b, 3b, 4b and 5b: AFM 2D images with scanning of 10x10 µm of CNM. 2c, 3c, 4c and 5c: surface roughness chart. Source: Adapted from Santos et al. (2008).

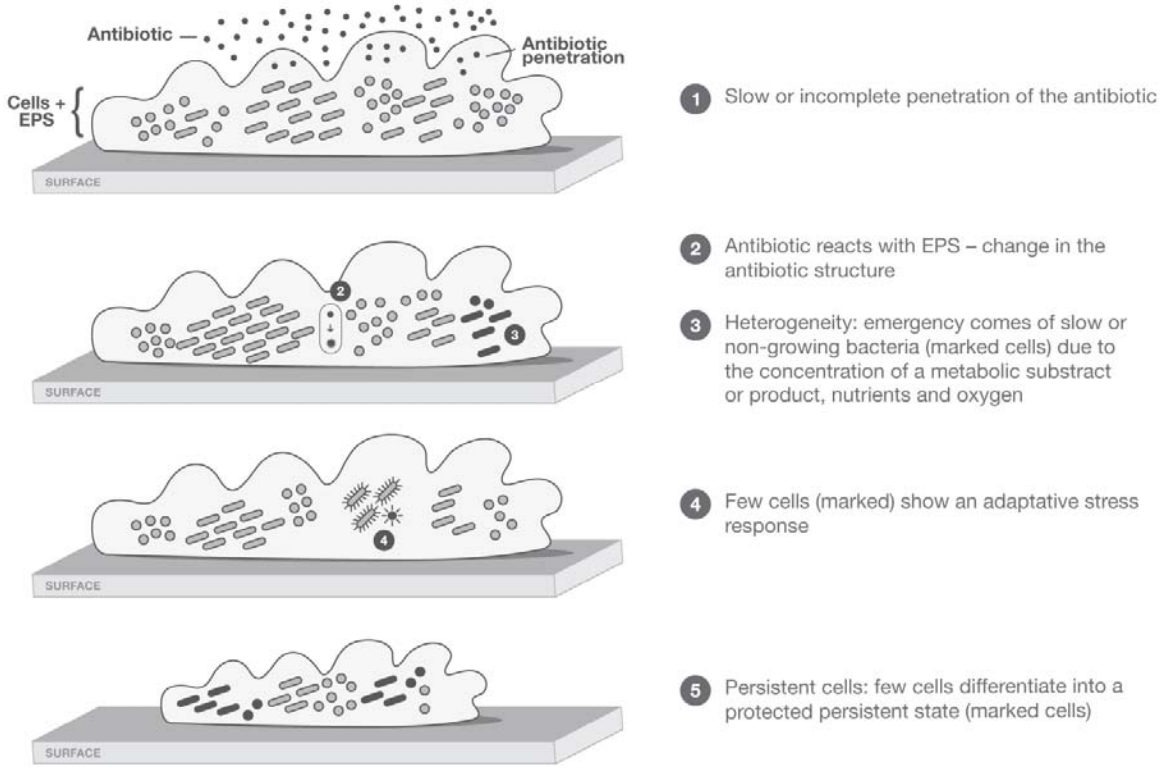

Figure 2. Mechanisms of antimicrobial resistance in bacterial biofilms.

possibly even if phagocytose occurs, macrophage production and release of reactive oxygen species is impaired (Jesaitis et al., 2003). Figure 2 shows the main mechanisms of resistance to antibacterial drugs in bacterial biofilms.

Antibiotics minimal inhibitory concentration (MIC) effective on biofilm-growing bacteria may be up to 1000-fold higher than that of planktonic bacteria (Hoiby et al., 2010). Biofilm-specific mechanisms are coordinately in a reversible and transient manner, contributing to the high levels of antibiotic resistance of these structures in a different pathway from the well-characterized intrinsic resistance mechanisms (for example, expression of anti-biotic-degrading enzymes, inducible decrease in antibiotic influx, inducible increase in antibiotic efflux and alteration in antibiotic target sites) employed by planktonic cells (Sun et al., 2013).

The biofilm EPS act as a barrier to delay the antibiotics diffusion into biofilms (Stewart, 2002) because the active substances may either react chemically with biofilm matrix components or attach to anionic polysaccharides (Sun et al., 2013).

Biofilms contain a small reversible subpopulation of so-called persister cells that adopt a slow-or nongrowing lifestyle through the emergence of small colony variants, and are highly tolerant to extracellular stresses, such as antibiotic treatment. Many antibiotics are less effective against slow- or non-growing cells when compared with fast-growing ones because these antibiotics target growth-specific factors; thereby, the slow growth rates of biofilm-growing cells will render them less susceptible to antibiotics (Sun et al., 2013). After antibiotic treatment, persister cells may survive, creating the reservoirs of cells that may regrow causing a recalcitrant chronic infection. When nutrients are limited in the media, bacteria become highly resistant to antibiotics; this phenomenon is called starvation (Nguyen et al., 2011). Starvation is found in biofilms as consequence of nutrient consumption by peripheral cells and reduced diffusion of oxygen and nutrients through biofilms. This condition induces the stringent response characterized as the repression of growth and division, with the stimulation of amino acid synthesis in order to promote bacterial survival (Chatterji and Ojha, 2001). The starvation response is a determinant of biofilm-specific antimicrobial resistance in *P. aeruginosa* (Nguyen et al., 2011).

Besides the aforementioned mechanisms, it was recently verified that inactivation of ethanol oxidation genes increases the sensitivity of *P. aeruginosa* biofilms to antibiotic treatment, indicating the contribution of ethanol oxidation to biofilm specific antibiotic resistance (Beaudoin et al., 2012).

Planktonic cells may exhibit multidrug efflux pumps that mediate antibiotic efflux leading to antibiotic resistance. Nine efflux pumps of *P. aeruginosa* have been shown to contribute to this organism's high intrinsic resistance to antibiotics (Mima et al., 2005). A *P. aeruginosa* efflux pump encoded by *PA1874-1877*, has been shown to contribute to the biofilm-specific antibiotic resistance of *P. aeruginosa* (Zhang and Mah, 2008).

A relevant factor in biofilm antibiotic resistance is the extracellular DNA within the biofilm matrix. This molecule can bind to and sequester cations, resulting in a cation-limited environment within biofilms that activates the two-component regulatory systems PhoP/Q and PmrA/B required for the expression of multiple antibiotic resistance genes in *P. aeruginosa* (Mulcahy et al., 2008).

LECTINS: CONCEPT AND CHARACTERISTICS

Based on lectins knowledge, Van Damme et al. (1996) defined it as proteins which have at least one non-catalytic domain that reversibly binds to carbohydrates, mono or oligosaccharides, and classified it into four types according to their structural characteristics: merolectins, hololectins, quimerolectins and superlectins. In 1996, the same authors introduced the class superlectins, which presents two carbohydrate binding sites, a significant different structure, and recognizes unrelated sugars. An example of this group is the lectin TxLC tulip-1 subunits, which has a specific site for mannose and one for N-acetyl-galactosamine, working completely independently (Van Damme et al., 1996).

In order to facilitate the use of lectins in glycobiology, Wu and colleagues (2009) classified the molecules according to their specificity for monosaccharides and oligosaccharides structures. These molecules can act as mediators of information in biological systems, and interact with glycoproteins, glycolipids and oligosaccharides (Gupta et al., 2010; Gomes et al., 2010).

Thus, lectins are a large group of proteins of structural heterogeneity which may differ in amino acid composition, apparent molecular weight, structure and number of subunits and also by whether or not related to metal ions or divalent cations (Cavada et al., 2001). These molecules have been used extensively in the physiology field, biochemistry and biomedical sciences. However, the true biological function of these proteins is not clear.

Some studies aimed to bring relevant issues on this topic (Rüdiger and Rouge, 1998; Lannoo and Van Damme, 2010), even for the family Leguminosae lectins with high primary sequence similarity, common functions could not be attributed, since some parameters, such specific carbohydrate, location and time of production are different (Carneiro, 2010).

Lectins in plants have important biological functions, such as protein reserves, defense and communication (Sharon, 1980; Cook, 1986; Van Damme et al., 1998). These molecules functions in plants are viewed from two perspectives: the lectin interacts with external sources, aggressors or symbionts (animals, bacteria or fungi), and another function in which the lectin plays a physiological role in the plant (Sharon and Lis, 1995).

Lectins with a high degree of similarity in amino acid sequence, secondary and three-dimensional structure are found in plants of Leguminosae family, thus revealing a well-defined taxonomic line (Cavada et al., 1993; Sharon and Lis, 1995). These lectins, generally comprise two or four subunits, that could be identical or different and with molecular weight of about 25-30 kDa. These subunits could be formed by a single polypeptide scaffold stabilized by non-covalent bonds like hydrogen bonds, electrostatic and hydrophobic interactions forming or not canonical dimmers (Vasconcelos, 2010). Lectins belonging to Diocleinae subtribe are tetramers composed by a mixture of intact subunits formed by a polypeptide chain of 237 amino acid residues and fragmented subunits, in which the same polypeptide chain is divided in two fragments (Chrispeels et al., 1986). Examples are the lectins ConA and ConBr, which have high structural similarity in amino acid sequences. The difference in crystalline structure between ConA and ConBr is only in two amino acids and neither of them is close to the carbohydrate binding site on both lectins. However, this difference makes ConBr structure more open than the Con A (Cavalcante et al., 2011).

Lectins have a variety of structural characteristics and are widely distributed in nature, been identified in fungi, bacteria, insects, animals, plants, as well as virus (Moreira et al., 1991). These molecules may be involved in various natural phenomena, among them the process of fertilization, embryogenesis, cell migration, organ formation and immune defense (Sharon and Lis, 2004). The imbalance of these processes may trigger the development of several pathologies (Sharon and Lis, 1989).

When Nowell (1960) described the mitogenic activity of *Phaseolus vulgaris* (PHA) lectin on human lymphocytes, an important new branch of research arose for the applicability of these molecules in biological systems. On cells surfaces there are carbohydrate molecules existing as glycoproteins, glycolipids and polysaccharides, and these molecules are directly involved in many cellular processes. The investigation of mechanisms involved in cell-cell interaction has emphasized the importance of carbohydrates in biochemical processes, viewed as energy-rich molecules or prosthetic elements (Carvalho, 2008).

Carbohydrates are essential elements for recognition in a wide variety of biological processes, in physiological and pathological conditions (Varki, 1993; Sharon and Lis, 1995). Thus, the fact of lectins often detect differences in

carbohydrates configuration, they would be powerful tools for this exchange of information between cells.

The use of lectins as biotechnological approaches are justified by a large number of scientific studies showing biological relevant activities related to these proteins (Kitada et al., 2010, Kimble et al., 2010; Singh et al., 2010; Cao et al., 2010). Among these biological activities, it is noted that lectins exhibit antibacterial activity (Alencar et al., 2005; Holanda et al., 2005, Wong et al., 2010), and lectins have ability to interfere with process of microbial biofilms formation (Teixeira et al., 2006, 2007, Oliveira et al., 2007; Islam et al., 2009; Cavalcante et al., 2011).

Various infections are started by lectin-carbohydrate interactions, such as cell adhesion and phagocytosis of *P. aeruginosa* (Imberty et al., 2004), *Neisseria gonorrhoeae* (Sharon, 2006), *Escherichia coli* (Firon et al., 1983), trypomastigote form of *Trypanosoma cruzi* (Silber et al., 2002) and promastigotes of *Leishmania major* (Sacks et al., 1985). Several of these pathogens establish mechanisms of a required attachment or adhesion to the host tissue or cells, otherwise, these microorganisms could be eliminated by the natural defense mechanisms of host, such as the airflow on respiratory system or urine excretory system (Sharon and Lis, 1993). In addition, proper adhesion of the pathogen provides better access to nutrient sources, facilitates the introduction of toxic substances in host tissue and even the penetration of the pathogen in these tissues (Karlsson, 1998).

Lectins action on biofilms mechanisms

The first report of inhibitory action of peptides in microorganisms, dated from 1942, refers to a protein obtained from wheat (Balls et al., 1942; Nakatsuji and Gallo, 2012). Lectins from higher plants have defense function against pathogens such as bacteria and fungi by immobilization and recognition of infectious microbial agents by binding, thus preventing the multiplication and subsequent colonization of the host plant (Etzler, 1986). Inhibition of bacteria growth and fungi by lectins, such as Amaranthus, has been previously reported in the literature (De Bolle et al., 1996). The concentrations used are considered higher than the concentration used in similar studies (Liao et al., 2003; Santi-Gadelha et al., 2006; Oliveira et al., 2007, 2008). However, Liao and colleagues (2003) tested the antimicrobial activity of plant and seaweed lectins using concentrations between 102 and 800 µg/mL and found that ConA and WGA from land plants did not inhibit any of the analyzed vibrios.

Lectins have antibacterial activity, and this effect (on Gram-positive and Gram-negative bacteria) occurs through interactions with bacterial cell wall components (Paiva et al., 2010). Santi-Gadelha and colleagues (2006), using electron microscopy, observed the presence of pores and severe disruption of bacterial membrane of Gram-positive, confirming the marked antimicrobial activity and pointing a possible mechanism of growth inhibition by lectins, since these pores formed in the membrane allows the output of the cell content (Terras et al., 1993; Oliveira et al., 2008).

By the genetic expression analysis of genes related to the *S. mutans* biofilm on *Canavalia maritime* lectin, Cavalcante et al. (2013) observed that although the mechanism of action of these lectins requires a better understanding, the results reported in that present article suggest that ConM acts by starting or interrupting intracellular signaling pathways that culminate with the lowest expression of genes associated with virulence and biofilm formation in *S. mutans*.

The carbohydrate binding sites on the bacterial surface probably have a key role in antibacterial activity, which makes it responsible for bacterial recognition. In a recent study, it was noted that differences in antimicrobial activity against *S. mutans* and *S. oralis* may be related to differences in the composition of surface carbohydrates characteristic of each bacteria. Almost all microorganisms express carbohydrates on its surface (Cavalcante et al., 2011). These carbohydrates may be covalently linked, as in teichoic acid linked to the peptidoglycan glycosylated or non-covalently linked, as in the capsular polysaccharides (Santi-Gadelha et al., 2006; Calderon et al., 1997).

The ability of lectins to form complexes with microbial glycoconjugates has stimulated its application as probes to whole cells, its mutants and numerous cellular constituents and metabolites. Microbial receptors for Concanavalin A have been described. For example, glycosylated teicoic acid found on the surface of various Gram-positive bacteria (Calderon et al., 1997) and neutral polysaccharides produced by members of the genera *Leuconostoc* and *Streptococcus* (Santi-Gadelha et al., 2006) can be sites to lectin binding. The development of high-affinity ligands able to selectively recognize a variety of different patterns in small oligosaccharides would be of significant interest as diagnostic and experimental tools for many bacterial infections. The selective binding of lectins to certain bacteria have been proposed for use in drug delivery of antimicrobial agents with the *Canavalia ensiformis* lectin having as point of action, *Streptococcus sanguis* and *Corynebacterium hofmannii;* and lectin of *Triticum vulgaris* targeting *Streptococcus epidermis* in *in vitro* experiments (Kaszuba et al., 1995).

CONCLUSIONS

Microbial biofilms formation and maintainability are directly linked to carbohydrate residues. These molecules mediate the adhesion of the bacteria to the surface

substrate (biotic or abiotic) for biofilm formation as well as, acting between microorganisms interaction to form cell aggregates. As molecules are able to bind specifically and selectively to carbohydrates, lectins have a crucial function in microbial biofilms studies, becoming a powerful tool to analyze glycidic structures of microbial origin aggregates.

The impact of bacterial resistance, provided by biofilm formation on human health encourages researches aiming to understand its mechanisms, as well as strategies to eradicate or minimize these communities' damages.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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