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Antibiofilm Activity of Lectins from Plants and Marine Invertebrates: A Comparative Study

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This paper has been dedicated to Professor Bishnu P. Chatterjee on his 80th birthday.

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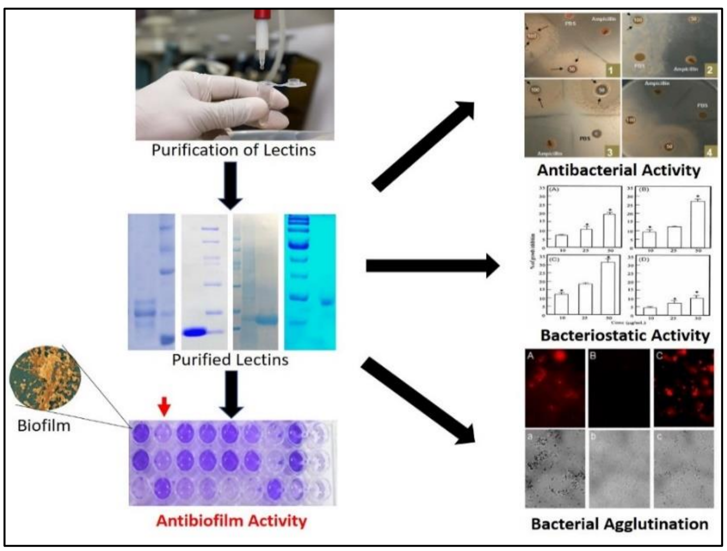
Graphical Abstract



Abstract

The present study includes seven lectins from plants and marine invertebrates, which were purified at our laboratories over the last 12 years. This work focused mainly on the antibiofilm activity of such lectins though other antibacterial activities have also been studied. The studied lectins showed specificity to galactose and *N*-acetyl aminosugars. Some lectins possessed broader sugar-binding specificity whereas others were very specific in nature. In most cases, lectins could not affect the planktonic growth of *P. aeruginosa* or *E. coli* despite of inhibiting the formation of biofilm in varying degrees. Besides the antibiofilm activity, different levels of antimicrobial activities against various pathogenic bacteria of the said lectins were compared with other lectins having similar properties. Revealing the molecular basis of these activities will be supportive to find their possible role in combined therapy with antibiotics.

Keywords: Lectins; Antibiofilm; Bacteriostatic; Bacterial agglutination; Marine invertebrates

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Abbreviations of Lectins

StL-20: *Solanum tuberosum* lectin; MytiLec-1: *Mytilus galloprovincialis* lectin; HOL-18: *Halichondria okadai* lectin; AKL-40: *Aplysia kurodai* lectin; OXYL: *Oxycomanthus japonicus* (present name: *Anneissia Japonica*) lectin; AGL: *Amaranthus gangeticus* lectin; TCLs: Tomato chitin-binding lectins; CiL-1 and CiL-2: *Codium isthmocladum* lectins; ALL: *Aplysina lactuca* lectin; CCL: *Chondrilla caribensis* lectin; ADEL: *Aplysia dactylomela* egg lectin

1. Introduction

In recent years, around 15 million people die of infectious diseases worldwide.[1,2] Apart from Coronavirus diseases, bacterial infections are the leading cause of death nowadays.[3,4] Severity of these infections depends on the multidrug resistance of various pathogenic bacteria like extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae, carbapenemase-producing *Klebsiella pneumoniae* (KPC) and methicillin-resistant *Staphylococcus aureus* (MRSA).[5-7] A number of virulence factors contribute to microbial drug resistance and production of biofilm by microbes is one of those.

Biofilm is the syntrophic consortium of certain microorganisms composed of extracellular polymeric substances like exopolysaccharides (EPS), lipids, proteins and nucleic acids.[8] It protects biofilm-producing bacteria through the development of a barrier between those and the environment, making those resistant to host immune system and antibiotic therapy.[9] Consequently, new therapeutic strategies and drug development are becoming important to combat multidrug resistance. Natural compounds like phytochemicals, purified proteins and peptides from plants or invertebrates showed significant antibiofilm activity that can augment the effectiveness of antibiotics if used combinedly.[10,11]

Lectins are proteins that bind with sugars or glycans and accomplish various functions in a number of cellular processes.[12,13] They are also involved in innate immunity and possess antimicrobial properties through their binding and interaction with microbial glycoconjugates.[14,15] A good number of lectins with antimicrobial activities have been isolated from plants and marine invertebrates, but not many lectins with antibiofilm activities were reported.[16-18] This study emphasized on the comparison of such lectins based on their sugar binding properties and antibiofilm potential.

2. Materials and Methods

2.1. Purification of lectins

Seven lectins have been isolated from Potato (*Solanum tuberosum*) tubers, Mediterranean mussels (*Mytilus galloprovincialis*), Japanese black sponge (*Halichondria okadai*), Crinoid Feather Star (*Anneissia Japonica*), Sea hare (*Aplysia kurodai*) eggs, Red Amaranth Seeds (*Amaranthus gangeticus*) and Tomato (*Solanum lycopersicum*) fruits, by different chromatographic techniques.[19-25]

2.2. Antibiofilm activity assay

Antibiofilm activity of different lectins was evaluated according to previously published methods.[19,25] In brief, both *E. coli* and *P. aeruginosa* were grown for 24 h and turbidity of bacterial cell suspensions was adjusted to 1.0 at OD_{640}. The bacterial suspension (50 µL) was mixed with same volume of purified lectins in 96-well microtiter plates and allowed to form the biofilm through incubation for 24 h at 37°C. The biofilm formed in wells was stained by 0.1% crystal violet for 10 min, washed with TBS to remove free dye and treated with 150 µL of 95% ethanol for 10 min. Absorbance values of each well were measured by an automated microtiter plate reader at 640 nm. Percentage reduction of biofilm formation resulting from lectin treated, relative to control samples, was calculated as:

$$\% \text{ Reduction of biofilm formation} = (1 - [OD_{640} \text{ experiment}/OD_{640} \text{ control}]) \times 100\%$$

2.3. Antimicrobial activity assay

Bactericidal and bacteriostatic activities of lectins were evaluated by the disc diffusion and titer plate assay.[25] Bacterial agglutination was checked according to the previously published works.[20-22]

3. Results and Discussion

In a span of 8 years (2014-2022), antibiofilm activity of seven lectins had been studied as well as their bactericidal, bacteriostatic and bacterial agglutination properties. StL-20, a chitin-binding lectin from potato tubers showed the maximum antibiofilm activity in terms of protein concentration (18% at 80 µg/mL). Out of three marine invertebrate lectins, MytiLec-1 (31% at 250 µg/mL) and HOL-18 (22% at 200 µg/mL) showed slightly higher activity than AKL-40 (22.5% at 250 µg/mL). OXYL was were comparatively more effective (40% at 200 µg/mL) whereas lectins from plant sources showed varying degrees of antibiofilm activity. Activity of AGL, the seed lectin was lesser (37% at 250 µg/mL) than OXYL but that of another chitin-binding lectin from the Solanaceae plant family, TCLs, was higher (53% at 250 µg/mL) (**Figure 1**).

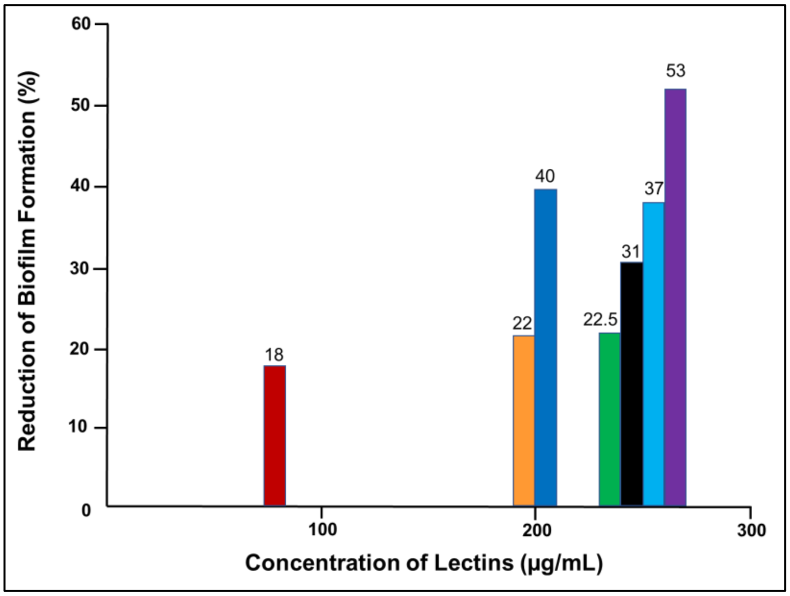

Figure 1. Antibiofilm activity of lectins from plants and marine invertebrates. Red, orange, blue, green, black, cyan and violet color denoted the activity of StL-20, HOL-18, OXYL, AKL-40, MytiLec-1, AGL and TCLs.

If we compare these activities with other marine lectins, similar degrees (40-50% at 250 μg/mL) of antibiofilm activity were found for green alga (*Codium isthmocladum*) lectins (CiL-1 and CiL-2) against *Staphylococcus aureus* and *S. epidermidis*.[26] ALL from marine sponge *Aplysina lactuca* possessed around 30% antibiofilm activity at a concentration of 250 μg/mL against *E. coli*.[27] On the other hand, another sponge lectin, CCL from *Chondrilla caribensis* could not inhibit the formation of biofilm by *E. coli* at 250 μg/mL. But it reduced the biofilm formation by *S. aureus* and *S. epidermidis* by 60% and 40%, respectively.[28] In case of plant lectins, a 2021-study reported a wider range of antibiofilm activity from six plant species including StL-20, which showed comparatively weaker activity than the others.[29]

Lectins used in this study showed specificity to their ligand sugars, galactose and *N*-acetylhexoamines. Affinity to these two certain sugars was common for other such lectins. Like AKL-40 and MytiLec-1, previously mentioned lectins (CCL, ALL, CiL-1 and CiL-2) had been reported to be galactoside-specific, in a broad sense.[26-28] Antibiofilm activity of another sea hare egg lectin (ADEL) from *Aplysia dactylomela* and a C-type lectin from snake venom (*Bothrops jararacussu*) was inhibited by galactose sugar, supporting these findings.[30-31] StL-20, TCLs, AGL, HOL-18 and OXYL bound to *N*-acetylhexoamines including *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetyl-lactosamine (**Table 1**).[19,21,22,24,25]

It became evident that despite of inhibiting the formation of biofilm by *P. aeruginosa* or *E. coli* in varying degrees, lectins could not affect the planktonic growth of those bacteria in most of the cases. AKL, AGL, CiL-1 and CiL-2 showed this property.[23,24,26] OXYL, CCL and ALL agglutinated the biofilm-forming bacteria, reduced biofilm formation but was unable to stop their planktonic growth.[22,27,28] HOL-18 inhibited the growth of *E. coli* but was inactive against *P. aeruginosa*.[21] StL-20 exerted both bactericidal and bacteriostatic activities against *P. aeruginosa* though only bacteriostatic activity against *E. coli* was observed for TCLs.[19,25]

Besides the antibiofilm activity, the aforementioned lectins possessed different levels of antimicrobial activities against various pathogenic bacteria (**Table 1**). ALL and CCL agglutinated *S. aureus* and *S epidermidis* cells, but both failed to inhibit their planktonic growth.[27,28] CiL-1 and CiL-2 showed similar property, which is also in line with our findings.[26]

Table 1. Sugar specificity and antimicrobial activity of lectins against different pathogenic bacteria

Lectins with their source	Sugar specificity	Antibiofilm activity against *E. coli/ P. aeruginosa*	Antimicrobial activity against *E. coli/ P. aeruginosa*	Antimicrobial activity against other pathogenic bacteria
TCLs (Tomato fruits)	*N*-acetyl-D-glucosamine	*E. coli*	Inhibited the growth of *E. Coli*	Bactericidal and bacteriostatic activity against *S. boydii* and *S. aureus*
AGL (Red Amaranth seeds)	*N*-acetyl-D-galactosamine	*E. coli*	No growth inhibition against *E. coli*, rather mitogenic growth (7–9%) was observed	Bacteriostatic activity against *S. boydii*, *S. dysenteriae* and *S. aureus*
AKL-40 (Sea Hare eggs)	D-galactose	*E. coli*	No bactericidal activity against *E. coli*	Bactericidal and bacteriostatic activity against *S. aureus*, *B. cereus* and *S. sonnei*
MytiLec-1 (Marine Mussel)	Globotriose (Galα1-4 Galβ1-4Glcβ)	*E. coli*	Agglutinated *E. coli* and inhibited their growth	Bactericidal activity against *B. cereus*, *S. sonnei*, *S. dysenteriae* and *S. boydii*
HOL-18 (Japanese Black Sponge)	*N*-acetyl-hexosamine	*P. aeruginosa*	Inhibited the growth of *E. coli* but exhibited negligible growth inhibition against *P. aeruginosa*	Bacteriostatic activity against *L. monocytogenes* and *S. boydii*
OXYL (Feather Star)	*N*-acetyl-lactosamine	*P. aeruginosa*	No growth inhibition but agglutinated *P. aeruginosa*	No growth inhibition against *L. monocytogenes*, *P. aeruginosa* and *S. boydii*
StL-20 (Potato tubers)	*N*-acetyl-D-glucosamine	*P. aeruginosa*	Bactericidal and bacteriostatic activity against *E. coli*, not tasted against P. aeruginosa	Bactericidal and bacteriostatic activity against *L. monocytogenes*, *S. enteritidis* and *S. boydii*

Lectins interact with glycan chains expressed on the cell wall of bacteria to agglutinate those as well as to exert diverse antimicrobial effects. Presence of *N*-acetyl sugar residues appears to be vital for the antibiofilm activity, so are the galactose residues. Some lectins like HOL-18 possessed broader sugar-binding specificity as its activity was inhibited by multiple sugars (*N*-acetyl D-glucosamine, *N*-acetyl D-mannosamine and *N*-acetyl D-muramic acid) whereas OXYL was very specific to *N*-acetyllactosamine, but not to lactose.[21,22] These lectins probably play protective roles in plants and marine organisms to eradicate microbes from their system, possibly through mechanisms triggered by lectin-glycan interactions.

4. Conclusion

This study attempts to shed light on the sugar specificity of lectins with antibiofilm properties. Summarizing such findings will provide a comprehensive perspective to further elucidate the molecular basis of their activity.

Authorship Contribution

Imtiaj Hasan, Sultana Rajia and A. K. M. Asaduzzaman performed the experiments. Yuki Fujii and S. M. A. Kawsar performed the analysis, interpreted and validated the data. Imtiaj Hasan and Yasuhiro Ozeki conceptualized, supervised and validated this work. Imtiaj Hasan also wrote the original draft along with reviewing and editing the manuscript.

Conflicts of interest

Authors declare no conflict of interest.

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