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ANTIBACTERIAL ACTIVITY OF A LECTIN ISOLATED FROM MARINE SPONGE *AXINELLA DONNANI*

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Abstract: This study is aimed to evaluate the ability of a new lectin isolated from marine sponge *Axinella donnani* to inhibit bacterial growth and biofilm formation. The antibacterial effect was studied using disc diffusion method. The results showed that the *Axinella donnani* lectin (ADL) exhibit a significant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus.* The activity of ADL against the biofilm formation was also tested by crystal violet assay. ADL significantly reduced the biomass of bacterial biofilm of *P. aeruginosa* and *S. aureus* in a dose dependent manner. These findings indicate that the lectin tested in this study may be natural alternative antimicrobial agents

Key words: *Axinella donnani,* lectin, Disc diffusion, Antibacterial activity, Antibiofilm activity.

INTRODUCTION

The continuous emergence of novel infections and the increasing threat of antibiotic resistance in both hospital settings and the food industry is now a major concern all over the world (Antelmann *et al.,* 2008; Chang *et al.*, 2015). Considering the diverse diseases and infectious agents that affect human species; researchers to date are focused on the development of biorecognition molecules with diagnostic and therapeutic potential to fight these pathologies. The key for efficient detection and treatment of diseases is in the biorecognition event. Thus, the production of drugs exploiting the efficiency of such biorecognition molecules from natural sources has become a prime focus of the pharmacological industry (Newman and Cragg, 2007). In this scenario bioactive agent from marine sources has emerged as a favorable candidate for the development of therapeutic agents including antibacterial drugs (Sunil *et al*., 2012).

Lectins are proteins which have at least one carbohydrate binding site that specifically and reversibly bind to mono- and oligosaccharides (Peumansand Van Damme, 1995). They are present in almost all organisms including plants, animals, viruses, bacteria and yeasts (Sharon, 2007). When considering drug discovery, lectins are one of the promising candidates, since carbohydrate structures such as, glycoproteins, proteoglycans and glycolipids have been implicated in several physiological and pathological functions including cell-cell communications and host-pathogen interactions (Wu *et al.*, 2009). They act as receptors for recognizing carbohydrate moieties present on the surfaces of pathogens and specifically gets bonded to them. Many lectins have also attracted significant attention for pharmacological applications, including antitumoral, antimicrobial, anti-HIV and anti-inflammatory activities (Ogawa *et al*., 2011).

Sponges, the evolutionarily oldest metazoan, belongs to the phylum Porifera, and are gaining considerable attention due to their ability to produce a variety of bioactive compounds having therapeutic potential (Muller *et al.*, 2001). More than two hundred bioactive products derived from sponges are reported yearly since the last decade (Hu *et al.*, 2011; Mehbub *et al.*, 2014). Interestingly, marine sponges exhibit a broad spectrum of biological activities. It has been suggested that lectins in marine sponges are involved in self-defense of the organism, since several lectins are able to recognize, agglutinate and inhibit the growth of bacterial cells and biofilms (Garderes *et al.*, 2015). Since the discovery of hemagglutinins in sponges by Dodd *et al.* (1968), there had been many

reports on the partial characterization of lectins from these oldest multicellular animals (Molchanova *et al.*, 2005). Till date approximately fifty lectins were isolated and biochemically characterized from phylum Porifera (Gardères *et al.*, 2015) in particular. The number of lectins which have isolated from marine organisms is considerably small as compared with the great variety of lectins isolated from plant origin.

There is a growing interest in evaluating the antimicrobial activity of lectins against bacteria of medical importance. The interaction between carbohydrates and lectins are involved in many physiological processes, and mediate a wide range of activities, including antimicrobial properties (Klafke *et al.*, 2013). Lectins can recognize and reversibly bind to carbohydrates on cell surfaces and interact with cell wall polysaccharides and/or glycoconjugates in the cell membrane (de Vasconcelos *et al.*, 2012). Furthermore, some lectins have shown to possess antimicrobial activity and are able to hamper the formation of biofilms (Islam *et al.*, 2008). Many lectins with antibacterial activity were purified from marine sponges such as *Suberites domuncula* (Schroder *et al.*, 2003), *Cliona varians* (Moura *et al.*, 2006) *Halichondria okadai* (Sarkar *et al.*, 2010), *Spheciospongia vesparia* (Fenton *et al.*, 2013) *Chondrilla caribensis*(Marques *et al.*, 2017) and *Stylissa flexibilis* (Hung *et al.*, 2018).

The purpose of this study is to evaluate the *in vitro* antibacterial activity of the lectin isolated from marine sponge *A. donnani* against clinically relevant microorganisms and its antibiofilm activity.

MATERIALS AND METHODS

Chemicals

Muller Hinton agar, Nutrient broth and Ampicillin were purchased from Himedia (Mumbai).Dimethylsulphoxide (DMSO) and Crystal violet were of the analysis grade commercially available.

Preparation of marine sponge *Axinella donnani* Lectin (ADL)

Lectin from the marine sponge *Axinella donnani* (ADL) was extracted with PBS buffer, fractionated by Ammonium sulphate precipitation and purified by DEAE- Cellulose ion exchange chromatography and gel filtration. The purified fractions which showed high activity were pooled and then freeze dried in a lyophilizer (Labconco-7740060).

Microorganisms

Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris* and *Klebsiella pneumoniae*) bacterial strains were provided by the Department of Biotechnology, University of Kerala.

Antimicrobial Activity

The *in vitro* sensitivity of the microbes to ADL was done by disc diffusion method (Bauer *et al.*, 1996). After preparation of the media, 10 ml of sterile nutrient broth was aseptically inoculated with the test culture organisms and incubated at 35±2°C for 18 hours. After incubation, the test cultures were applied on air dried nutrient agar plates using a sterile glass spreader. Using a clean forceps, the sterile discs loaded with the lectin was placed on the surface of Muller Hinton Agar plates seeded with the test bacterial strains. Gentamycin discs were used as the control. The plates were then incubated at 35±2°C for 24 hours. The zone of bacterial growth inhibition was observed and its diameter was measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by a broth micro dilution method proposed by Novy *et al.*, (2015). Briefly, 100 µL of Nutrient Broth (Himedia) plus different concentrations of ADL was prepared and transferred to each microplate well to obtain dilutions ranging from 1.0 to 25mg/mL Then, 10 µL of a fresh culture of test organisms was added. Microplates were incubated at 37°C for 24 h. The reading of results are made manually using a black card and electronically with ELISA plate reader. MIC was defined as the lowest concentration of lectinwhich restricted the visible growth of microorganism tested.

Antibiofilm activity

The effect of ADL on biofilm-forming bacteria such as Gram-negative *P. aeruginosa* and gram positive *S.aureus* was tested according to Stepanovic

et al (2000) with modifications. NB broths containing the bacterial biofilms were established in 96 wells plates by incubating them for 72 hours. Different concentrations of ADL ranging from 7.8 µg/ml to 1000µg/ml were treated with the biofilms and incubated at 37ºC for 24 hours. For quantification the wells were washed with phosphate buffered saline (PBS) and stained with 1% crystal violet for 10 min. The untreated biofilm was taken as control. The stained adhered cells were removed by using micropipetting method, washed with PBS and dissolved in 300µl DMSO. The absorbance was read at 600nm (Agilent Cary 60, USA).

Statistical analysis

All the experiments were performed in triplicate (n=3). The data were expressed as mean ± standard deviation.

RESULTS

Antimicrobial activity

The trend of antibacterial activity of ADL is given in Table.1. ADL showed a high antibacterial activity against gram negative organisms such as *E.coli, K. pneumoniae* and *P.aeruginosa*. ADL showed less activity against gram positive bacteria except *S. aureus.*

ADL exhibited identical bactericidal activity against the tested gram-negative bacteria. The MIC exhibited by ADL was in the range of 500-1000 µg/ml.

Antibiofilm activity

The results of the activity of ADL on biofilm formation are shown in Fig. 1 & 2. The data indicates that ADL caused a significant reduction in the total biomass of biofilm produced by *P. aeruginosa* and *S. aureus*. Biofilm is reduced proportionally to the increase in lectin concentration.

Table 1. Antibacterial activity of *Axinella donnani* lectin (ADL)

Name of bacteria	Percentage inhibition of bacterial growth (1mg/ml ADL)(mm) (mean ± SD)	Percentage inhibition of bacterial growth Ampicillin (250 µg)(mm) (mean ± SD)
E. coli	11.33 ± 1.15	27 ± 1.73
K. pneumoniae	11.67 ± 1.54	21.33 ± 1.15
P. aeruginosa	10.67± 0.57	23 ± 1.73
S. marcescens	Nil	20±2
P. vulgaris	Nil	18.33 ± 1.53
B. subtilis	Nil	22.67 ± 1.15
S. aureus	13 ± 1.73	24 ± 1.73
S. pyogenes	Nil	14 ± 1.73

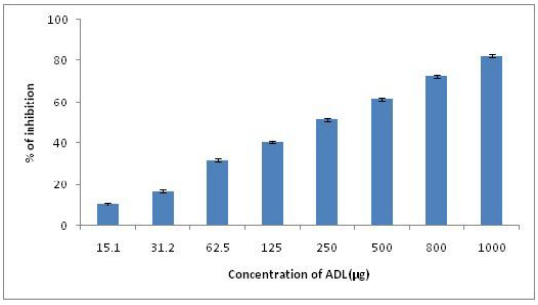

Fig. 1. Antibiofilm activity of ADL against *Pseudomonas aeruginosa*

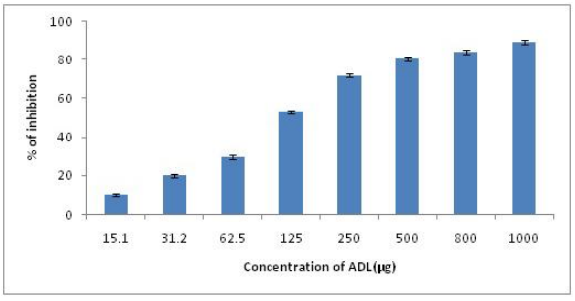

Fig. 2. Antibiofilm activity of ADL against *Staphylococcus aureus*

DISCUSSION

This study investigated the effect of ADL, a new lectin purified from marine sponge *Axinella donnani* on bacterial growth and biofilm formation. ADL (1mg/ml) exhibited a significant antibacterial effect on the gram-negative bacteria, *E. coli, P. aeruginosa, K. pneumoniae* and gram positive *S. aureus*. The minimum inhibitory concentration (MIC) of ADL was found to be in the range of 500-1000 µg/ml (Table 2). Our results are in agreement with certain previous reports, which mention greater activity of lectin towards Gram-negative microorganisms compared to Gram-positive microorganisms (Medeiros *et al.*, 2010; Pajic *et al.*, 2002; Hung *et al.*, 2018). These differences can probably be attributed to the structural and compositional differences in the cell wall and membranes. The mechanisms by which lectins exert their activity are not well described, but it is believed that their antibacterial activity against gram-positive and gram-negative bacteria occurs through interactions of the lectins with components of the bacterial cell wall (Paiva *et al.*, 2010). Lectins may recognize and bind to glycans on cell surfaces, cell wall polysaccharides and interact with bacterial lipopolysaccharides (LPS) or with the extracellular matrix of microorganisms (Miki *et al.*, 2012; Purish *et al.*, 2013; Shin, *et al.*, 2000; Strathmann *et al.*, 2002). It has been reported that lectins are able to interact with bacterial LPS (Kawsar *et al.*, 2009; Medeiros *et al.*, 2010). Furthermore, according Gardères (2015) the ability of sponge lectins to bind specific carbohydrates in bacterial cells could potentially be used to develop new antimicrobial agents.

Microbial biofilms are communities of bacteria, enclosed in a self-produced polymeric matrix that contains exopolysaccharides, teichoic acids, enzymes and extracellular DNA (Hall-Stoodley *et al.*, 2004; Ciofu *et al.*, 2010). Previous studies have demonstrated that bacteria inside biofilms can be upto 1000 times more resistant to antibiotics than free living bacteria. Moreover, biofilms have been found to be involved in many chronic diseases such as cystic fibrosis, periodontal disease and urinary tract infections (Mulcahy *et al.*, 2014). Compared to free living organisms biofilms are less sensitive to antibiotics, so the discoveries of new agents that are able to eradicate such biofilms are critical. Indeed, in recent years lectins have been demonstrated to be active agents against bacterial biofilms. In this study we observed that ADL significantly reduced the biomass of the biofilms produced by *P. aeruginosa* and *S. aureus* in a concentration dependent manner (Fig. 1 & 2). *P. aeruginosa* represents a commonly used biofilm model organism since it is involved in different types of biofilm-associated infections (Rybtke *et al.*, 2015). The ability of some lectins to inhibit biofilm formation in gram-negative bacteria may result from interactions between the lectin and LPS, affecting the adherence of these bacterial cells. These findings indicate that ADL tested in this study may have the potential to be developed as an alternative to conventional antimicrobial agents. However, further studies are required to better understand the functional efficacy of this lectin.

Table 2. Minimum Inhibitory Concentration (MIC) of *Axinella donnani* Lectin (ADL)

Name of bacteria	MIC(µg/ml)
E. coli	1000
K. pneumoniae	500
P. aeruginosa	500
S. aureus	500

CONCLUSION

Axinella donnani is a marine sponge seen in the peninsular coast of India. This study investigated the antibacterial potential of a newly isolated lectin ADL from *A. donnani* against pathogenic bacteria. The antibiofilm properties of the lectin was also analyzed. This study has demonstrated that ADL exhibited a considerable antibacterial effect particularly against gram negative organisms in a dose dependent manner. A significant reduction in biomass of the biofilms produced by *P. aeruginosa* and *S. aureus* were also observed after the application of ADL. These preliminary findings regarding the antimicrobial property of ADL may be helpful for the development of a potent antimicrobial agent for treating infections caused by some clinical pathogens. However further studies are needed for getting a better understanding of the exact mechanisms.

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