

Biological Effects of a Carbohydrate-Binding Protein from an Annelid, *Perinereis nuntia* Against Human and Phytopathogenic Microorganisms

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Abstract—Lectins have a good scope in current clinical microbiology research. In the present study evaluated the antimicrobial activities of a D-galactose binding lectin (PnL) was purified from the annelid, *Perinereis nuntia* (polychaeta) by affinity chromatography. The molecular mass of the lectin was determined to be 32 kDa as a single polypeptide by SDS-PAGE under both reducing and non-reducing conditions. The hemagglutinating activity of the PnL showed against trypsinized and glutaraldehyde-fixed human erythrocytes was specifically inhibited by D-Gal, GalNAc, Gal β 1-4Glc and Gal α 1-6Glc. PnL was evaluated for *in vitro* antibacterial screening studies against 11 gram-positive and gram-negative microorganisms. From the screening results, it was revealed that PnL exhibited significant antibacterial activity against gram-positive bacteria. *Bacillus megaterium* showed the highest growth inhibition by the lectin (250 μ g/disc). However, PnL did not inhibit the growth of gram-negative bacteria such as *Vibrio cholerae* and *Pseudomonas* sp. PnL was also examined for *in vitro* antifungal activity against six fungal phytopathogens. PnL (100 μ g/mL) inhibited the mycelial growth of *Alternaria alternata* (24.4%). These results indicate that future findings of lectin applications obtained from annelids may be of importance to life sciences.

Keywords—*Perinereis nuntia*, Lectin, Inhibition zone, Mycelial growth.

I. INTRODUCTION

CARBOHYDRATE binding proteins (lectins) have ability to agglutinate erythrocytes, other normal and cancer cells and microorganisms. Lectins can be found in all kingdoms of organisms from viruses through bacteria and plants to higher animals. Lectins bind monosaccharides, oligosaccharides and glycoproteins reversibly and with high specificity. Each lectin molecule typically contains two or more carbohydrate-combining sites which are usually di- or

polyvalent. When lectins interact with carbohydrates, they cross-link the glycoproteins and cause subsequent precipitation or agglutination [1]. In invertebrates, lectins have been suggested to participate in the innate immune response by inducing bacterial agglutination or by acting as opsonins to enhance phagocytosis by coelomocytes [2]. Besides their roles in cell recognition and host defense, lectins have been used as probes to determine the sugar composition of glycan and glycoconjugates such as bacterial lipopolysaccharides, cell surface glycoproteins and glycolipids [3]. Cell lysates and cell-free plasma of several invertebrates also expressed antibacterial activity, though the activity of the latter may be possible due to small antimicrobial proteins [4]. These results strongly support the contention that invertebrates possess 'immune-like' defense mechanisms.

Annelida comprises an ancient and ecologically important animal phylum with over 16,500 described species and members are the dominant macrofauna of the deep sea. Traditionally, two major classes are distinguished: Polychaeta (marine worms) and Clitellata (earthworms, leeches). Polychaeta is a large class in phylum annelid. They have segmented body cavities (coelom) filled by coelomocytes and well developed organs such as sensory nervous system, muscled body wall, and gut. In recent years, marine organisms have become attractive for lectinologists as new origins and sources of unusual lectins. A number of lectins which recognize D-galactose, N-acetyl-D-galactosamine, and N-acetyl-D-glucosamine are isolated from annelids [5-11]. Lectins have attracted the attention of numerous researchers by virtue of the potentially exploitable activities that they manifest including anti-proliferative, antitumor, immunomodulatory [12-14], antifungal [15], antiviral [16] and anti-insect [17] activities.

Gram-positive bacteria are those that are stained blue or violet by gram staining. This is in contrast to gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counterstain (safranin) and appearing red or pink. Gram-positive organisms are able to retain the crystal violet stain because of the high amount of peptidoglycan in the cell

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wall. Gram-positive cell walls typically lack the outer membrane found in gram-negative bacteria. The cell wall of virtually all bacteria consists of a rigid peptidoglycan layer that is either overlaid by an outer lipopolysaccharide (LPS) layer in gram-negative bacteria or remains exposed on the surface of gram-positive bacteria. Peptidoglycan is a polymer of alternating *N*-acetylglucosamine (GlcNAc or NAG) and *N*-acetylmuramic acid (NAM) units connected by short pentapeptides. The β -1,4-glycosidic bond of the *N*-acetylglucosamine, *N*-acetylmuramic acid peptidoglycan backbone can be hydrolyzed by lysozyme (muramidase; mucopeptide *N*-acetylmuramoylhydrolase), a ubiquitous enzyme involved in innate immune reaction of numerous animal species, including annelids [18, 19]. It has been reported that many lectins from marine invertebrates show antibacterial activity. The lectin from the horse mussel *M. modiolus* [20] exhibited strong antibacterial activity against tested *vibrio* strains. The gigalectin H and gigalectin E lectins from oyster, *Crassostrea gigas* acted as opsonins to stimulate *in vitro* phagocytosis of the marine bacterium *Vibrio anguillarum* [21]. T-antigen binding lectin purified from sea cucumber showed strong broad spectrum antibacterial activity against both gram-positive and gram-negative bacteria [22]. A novel antibacterial peptide was purified from the earthworm, *Eisenia foetida* [23]. On the other hand, a 10 kDa cadmium-binding protein was purified from the coelomic fluid of polychaeta, *Nereis diversicolor* and it showed antibacterial activity against *Escherichia coli* [24]. The antimicrobial peptide, hedistin was also purified from the same species and possessed activity against a large spectrum of bacteria including *Staphylococcus aureus* and *Vibrio alginolyticus* [25]. Lectins from the marine cyanobacterium, *Oscillatoria agardhii* also showed strong anti-human immunodeficiency virus (HIV) activity [26]. In a similar way, two polychaeta lectins from Chaetopteridae and Sabellidae inhibited the syncytium formation of HIV-1 infected C8166 cells [7, 8]. They are a 30 kDa polypeptide recognizing D-galactose and disulfide-bounded quartermer consisting of 12.7 kDa polypeptides recognizing *N*-acetyl-D-galactosamine, respectively.

Perinereis nuntia (Family: Nereididae) lives in the sand of tidal zones and the sandy bottom of shallow sea throughout Japan, Micronesia, and Australia. A variation, *vallata* reproduces at the tideland. *P. nuntia* var. *vallata* are artificially bred in Japan as fish feed. We previously determined the primary structure of PnL from *P. nuntia* var. *vallata* and evaluated its glycan-binding properties by frontal affinity chromatography [27]. Herein we report on our evaluation of the antibacterial and antifungal activities of PnL against some human- and phytopathogens.

II. MATERIALS AND METHODS

A. Materials

A lactosyl-agarose column was purchased from Seikagaku Kogyo Co. Ltd., Japan. A standard protein marker mixture

(Daiichi-III) for SDS-PAGE was purchased from Daiichi Pure Chem. Co. Ltd., Japan. A bicinchoninic acid (BCA) kit was purchased from Pierce Co. Ltd., USA. Superdex 75, Sephadex G-75 were obtained from GE Healthcare, USA. Agar, dextrose, peptone, beef extract were purchased from Merck Ltd., India and BDH Ltd., Bangladesh and were of the highest purity grade. The marine worm *P. nuntia* was supplied from a fishing shop in Yokohama City, Japan. Worms were stored at -80°C or used after collection according to the situation.

B. Affinity Purification of Lectin (PnL)

PnL, D-galactose specific lectin was purified from the marine worm *Perinereis nuntia* by affinity column chromatography [27]. Briefly, two hundred grams (wet weight) of worms were disrupted to paste with a razor blade and homogenized with 10 volumes (w/v) of Tris-buffered saline (TBS) (10 mM Tris(hydroxymethyl) aminomethane-HCl, pH 7.4, containing 150 mM NaCl) containing 2 mM of a protease inhibitor mixture. The homogenates were centrifuged at 14,720 g in 500-mL centrifuge bottles for 1 h at 4°C with a Suprema 21 centrifuge equipped with an NA-18HS rotor (TOMY Co. Ltd., Japan). The crude supernatant was centrifuged again at 27,500 g for 1 h at 4°C and the precipitate was homogenated again with 3 volumes (w/v) of TBES (TBS containing 10 mM EDTA) containing 50 mM galactose for over night at 4°C. It was centrifuged at 27,500 g for 1 h at 4°C and the supernatant was dialyzed against TBS till free from galactose. The crude supernatant was applied to an affinity column of lactosyl-agarose (10 mL) being fitted with a Sephadex G-75 pre-column (5 mL). After applying the extracts, the lactosyl-agarose column was extensively washed with TBS. The lectin was eluted with 50 mM galactose in TBS and 1 mL fractions were collected by an auto-fraction collector. Each chromatography step during washing and elution was monitored using a UV monitor (ATTO Co. Ltd., Japan) by the measurement of the absorbance at 280 nm.

C. Hemagglutinating Activity

The hemagglutination assay was performed using 1% (w/v) trypsinized and 0.25% glutaraldehyde-fixed rabbit and human erythrocytes as described previously [28]. Erythrocytes were suspended at a concentration of 1% (w/v) in TBS. In the general assay, 20 μ L each of TBS, TBS containing 1% Triton X-100, and erythrocytes were added to 20 μ L of the two times-serially-diluted lectin with TBS in 96 well V-shape titer plates for 1 h. The hemagglutination activity of the lectin was expressed as the titer defined as the reciprocal of the highest dilution giving positive hemagglutination. To determine the sugar binding specificity of the lectin, 20 μ L of each of the sugar (200 mM) and the glycoprotein (5 mg/mL) was serially diluted with TBS and added to lectin with the titer of 16, 1% Triton X-100, and erythrocytes in 96 well V-shape titer plates for 1 h incubation. The minimum inhibitory sugar

concentration against the lectin was expressed as negative activity.

D. Electrophoresis

The molecular mass of the polypeptide was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Purified lectin was mixed with an equal amount of sample buffer (20 mM Tris-HCl, pH 6.8; 0.2% SDS, and 20% glycerol) and then heated at 70°C for 15 min. Aliquots of 30 µL were applied to the well of a mini-slab gel (gel size: 80 mm × 100 mm with 1 mm thickness; 12% and 5% polyacrylamide were used in separation and upper gels, respectively, constant current at 30 mA for 1 h) according to a previous report [29]. The following polypeptides were used as molecular mass markers; phosphorylase b (M_r 94 kDa), bovine serum albumin (M_r 66 kDa), ovalbumin (M_r 42 kDa), carbonic anhydrase (M_r 30 kDa), trypsin inhibitor (M_r 20 kDa), and lysozyme (M_r 14 kDa). After SDS-PAGE, the gel was stained with 0.1% (w/v) Coomassie Brilliant Blue (CBB) R-250 in 40% (v/v) and 10% acetic acid (v/v) followed by discoloration by excessive staining with 40% methanol and 10% acetic acid.

E. Gel Permeation Chromatography

The purified lectin was dissolved in 2.5% glycerol and subjected to gel permeation chromatography (GPC) utilizing a Superdex 75 column (1.0 × 65 cm) connected to an FPLC system (GE Healthcare, USA) in the presence of 50 mM galactose containing TBS. The elution time of the lectin from the column was detected by UV at an absorbance of 280 nm. Bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa) and myoglobin (17 kDa) were used as standard molecular marker.

F. Protein Content

Protein concentrations were determined using BCA protein assay kit [30, 31] with bovine serum albumin as the standard by measuring absorbance at 562 nm with spectrophotometer ND-1000 (Nano Drop Tech. Inc., USA).

G. Microorganisms

The bacterial and fungal strains used in this study were obtained from the Microbiology Laboratory, Department of Microbiology, University of Chittagong, Bangladesh. Gram-positive bacterial strains were *Bacillus subtilis* BTCC 17, *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18 and *Staphylococcus aureus* ATCC 6538 and Gram-negative bacterial strains were *Salmonella typhi* AE 14612, *Salmonella paratyphi* AE 146313, *Shigella dysenteriae* AE 14396, *Shigella sonnei* CRL (ICDDR,B), *Escherichia coli* ATCC 25922, *Vibrio cholerae* (CRL (ICDDR,B) and *Pseudomonas sp.* CRL (ICDDR,B). The fungal pathogens were *Alternaria alternata* (Fr.) Kedissler, *Botryodiplodia theobromae* Pat., *Curvularia lunata* (Wakker) Boedijin, *Colletotrichum corcori*

Ikata (Yoshida), *Fusarium equiseti* (Corda) Sacc., and *Macrophomina phaseolina* (Tassi) Goid.

H. Medium and Culture

Standard NA medium was used for growing bacterial strains throughout the work. A 20 g of agar powder, 5 g of peptone, 3 g of beef extract and 0.5 g of NaCl was added slowly to 1000 mL water and the solution was mixed thoroughly with a glass rod. After 10 minutes of boiling, the medium was transferred into 500 mL conical flask and flask was closed with a cotton plug. The medium was autoclaved for 15 minutes at 121°C and 15 psi and ready to use bacterial culture. Older cultures were transferred to freshly prepared NA slants separately for each species via sterilized bacterial loop. In such a way, four test tubes were freshly prepared for each bacterial pathogen. These test tubes of inoculated slants were incubated at 35±2°C in incubator for 18-24 hours and each culture was used throughout for antibacterial screening studies. For preservation of the stock culture, one set of culture slants were kept in polythene bag, properly tied and preserved at 10°C.

I. Growth Inhibition Assay by Human Pathogens

The *in vitro* growth inhibition assay against bacteria by PnL was carried out by the disc diffusion method [32]. In this method sterilized paper discs of 4 mm in diameter and petridishes of 150 mm in diameter were used throughout the experiment. The autoclaved Mueller-Hinton agar medium, cooled to 45°C, was poured into sterilized petridishes to a depth of 3 to 4 mm and after solidification of the agar medium; the plates were transferred to an incubator at 37°C for 15 to 20 minutes to dry off the moisture that develops on the agar surface. The plates were inoculated with the standard bacterial suspensions (as of McFarland 0.5 standard) by help of sterilized glass and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were treated separately with 20µL (250 µg/disc) from 5% PBS solution of lectin using a micropipette, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A control plate was also maintained in each case without any test material. These plates were kept for 4-6 hours at low temperature and the test materials diffused from disc to the surrounding medium by this time. The plates were then incubated at 35±2°C for 24 hours to allow maximum growth of the organisms. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter. Each experiment was repeated thrice. Galactose was used as negative control. All the results were compared with the standard antibacterial antibiotic ampicillin (20 µg/disc), (BEXIMCO Pharma Bangladesh Ltd.).

J. Mycelial Growth Inhibition Assay by Phytopathogens

The *in vitro* antifungal activity of the annelid lectin was determined by the poisoned food technique [33, 34]. PDA medium was used for the culture of fungi. A required amount of

PDA was taken in conical flasks separately and was sterilized by autoclave (121°C, 15 psi) for 15 minutes. PnL (in PBS solution) was mixed with sterilized melted PDA medium to have 100 µg/mL PDA and this was poured (about 20 mL/plate) in sterilized petridishes. At the center of each plate, 5 days old fungal mycelial block (4 mm in diameter) was inoculated and incubated at 27°C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was measured after 3-5 days of incubation in triplicate. The average of two measurements was taken as mycelial colony diameter of the fungus in mm. All the antifungal results were compared with the standard antifungal antibiotic Nystatin (100 µg/mL PDA). Galactose was used as negative control. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

$$\% \text{ Inhibition} = (C-T/C) \times 100$$

Where, C = diameter of the fungal colony in the control petridish and T = diameter of the fungal colony in the treated petridish.

III. RESULTS AND DISCUSSION

The dialyzed crude supernatant obtained from the precipitate of annelid by homogenizing with 50 mM galactose in TBS showed strong hemagglutinating activity. Since it was inhibited by lactose or galactose in TBS, it was applied on a column packed by lactosyl-agarose. *P. nuntia* galactose-binding lectin was specifically eluted from the lactosyl-agarose column by the application of crude extraction by 50 mM galactose in TBS (Fig. 1). The purified lectin that was subjected to SDS-PAGE, showed that a 32 kDa lectin was isolated under both reducing and non-reducing conditions (Fig. 2). The molecular mass of the lectin was shown to be a 32 kDa polypeptide in the presence or absence of 10 mM 2-mercaptoethanol (Fig. 2, column: NR and R). Generally, 2.6 mg of PnL was purified from 1 kg (wet weight) of the worms (Table 1). The purified lectin showed strong hemagglutinating activity against trypsinized and glutaraldehyde-fixed human erythrocytes (Table 2). The relative molecular mass of the native PnL was estimated by GPC on a Superdex 75 column connected to an FPLC system using TBS containing 50 mM galactose. PnL eluted at the relative molecular mass of 32 kDa (Fig. 3), indicating that PnL is monovalent polypeptide in the buffer.

In vitro, antibacterial susceptibility by PnL was assayed against eleven human pathogenic bacteria and compared to that of the antibacterial antibiotic, ampicillin. The results of the sensitivity test are presented in Table 1 and 2. PnL (250 µg/disc) exhibited a strong antibacterial effect against gram-positive bacteria such as *Bacillus megaterium*. The diameter zone of inhibition caused by the addition of PnL was significantly effective against *Bacillus megaterium* and *Bacillus subtilis* (11 and 6 mm, respectively; Table 3).

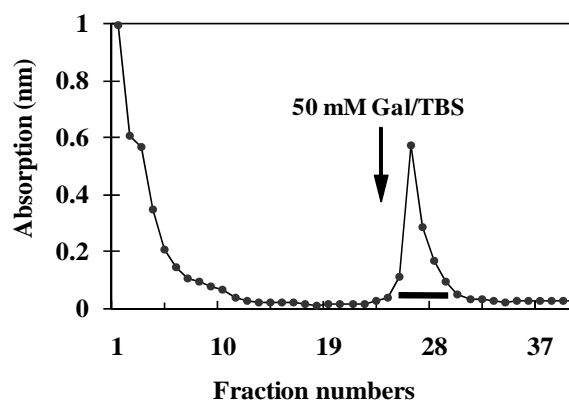


Fig. 1 Affinity purification of annelid lectin PnL. Dialyzed extraction obtained from galactose/TBS extract of *P. nuntia* was applied to a lactosyl-agarose column equilibrated with TBS. The column was extensively washed with TBS and the lectin was eluted with 50 mM galactose in TBS (arrow). Closed circles show the absorbance at 280 nm. The column bound fractions shown by the bar were collected and designated as purified lectin.

However, the lectin inhibited less, *Bacillus cereus* and *Staphylococcus aureus*. On the other hand, PnL did not inhibit growth against gram-negative bacteria and only *Salmonella typhi* exhibited any sensitivity to the lectin (Table 4). The ampicillin control inhibited growth in all cases. Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the lectin as compared to gram-negative bacteria. They belong to the same genus within *Bacillus*. The glycomics approach to determine the structure of surface glycans of bacteria will provide us with more useful information to prevent the disease using lectins. The glycan binding profile of PnL has been analyzed by FACT [27] and it specifically recognizes branched complex type N-linked oligosaccharides having lactosamine (Galβ1-4GlcNAc) or sialyllactosamine (Siaα2-3 Galβ1-4GlcNAc) chains which were seen in fetuin and tyroglobulin.

Recently, a lipopolysaccharide-binding lectin purified from seeds of *Eugenia uniflora* had shown similar antibacterial activity with PnL, as it effectively inhibited the growth against gram-positive bacteria such as *Bacillus megaterium* [35].

TABLE I
PURIFICATION STEPS OF GALACTOSE-BINDING LECTIN FROM ANNELID, *PERINEREIS NUNTIA*

Fraction	Titer (HU)	Total activity ^a	Specific activity ^b	Purification ratio (Fold)
Crude extract	512	102400	1.6	1
Affinity purification	2048	20480	1137	711

^aTotal activity is shown by Titer × volume.

^bSpecific activity was shown by titer/mg of protein.

Additionally, a β -galactoside binding pearl shell lectin purified from the marine bivalve, *Pteria penguin* had shown very similar antibacterial activity with PnL, as it effectively inhibited the growth against both gram-positive and gram-negative bacteria [36]. Lastly, rhamnose-binding steelhead trout (*Oncorhynchus mykiss*) egg lectin inhibited the growth of gram-positive and gram-negative bacteria by recognizing lipopolysaccharide or lipoteichoic acid [37] similarly to PnL.

Structural characterization of some lectins revealed the presence of specific binding sites which react with carbohydrate exposed on the surface of microorganisms, making possible the identification of pathogenic bacteria based on the selective agglutination between lectins and bacteria [38, 39]. Lectins are used to analyze carbohydrates presented on the cell walls of gram-positive and gram-negative bacteria because they contain various complex carbohydrates containing

TABLE II
AGGLUTINATION OF DIFFERENT BLOODS BY THE LECTIN

Blood sources*	Titer (HU)
Human	
Type A	2048
Type B	1024
Type O	2048
Rabbit	1024

*Trypsinized and glutaraldehyde fixed erythrocytes were used.

TABLE III
GROWTH OF INHIBITORY EFFECTS BY THE PURIFIED LECTIN
AGAINST GRAM-POSITIVE BACTERIA

Name of bacteria	Diameter zone of inhibition in mm	
	Lectin (250 μ g/disc)	Ampicillin* (20 μ g/disc)
<i>Bacillus megaterium</i>	11 \pm 1	18 \pm 1
<i>Bacillus subtilis</i>	6 \pm 1	19 \pm 1
<i>Bacillus cereus</i>	4 \pm 1	20 \pm 1
<i>Staphylococcus aureus</i>	4 \pm 1	16 \pm 1

*Standard antibacterial antibiotic, Statistical analysis (RBD) at 1% level, organisms significant (F value 183.5), replica significant (F value 6.95).

teichoic acid, peptidoglycan and lipopolysaccharides [40].

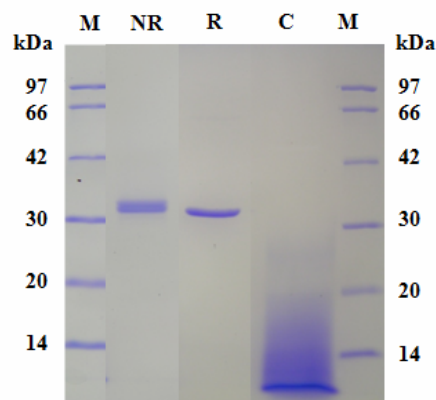


Fig. 2 SDS-PAGE pattern of PnL. Crude extract (C) and PnL were subjected onto SDS-PAGE under non-reducing (NR) and reducing (R) conditions. Standard marker proteins (M) were used as follows, phosphorylase b (97 kDa); bovine serum albumin (66 kDa); ovalbumin (42 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20 kDa) & lysozyme (14 kDa).

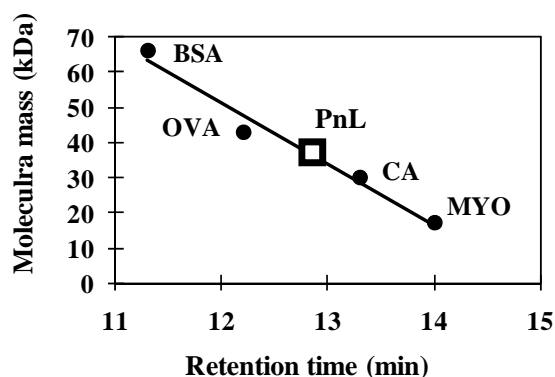


Fig. 3 Determination of molecular weight by gel permeation chromatography of PnL. PnL (20 μ g) was separated on Superdex 75 column using FPLC system at a flow of 0.5 mL/min (chart speed is 0.5 cm/mL). Calibration line for the determination of molecular weight of PnL was determined using standard molecular mass marker proteins as bovine serum albumin (BSA; 66 kDa), ovalbumin (OVA; 42 kDa), carbonic anhydrase (CA; 30 kDa) and myoglobin (MYO; 17 kDa).

In vitro antifungal activity by PnL was determined against six phytopathogenic fungi with the antifungal antibiotic nystatin as a positive control. Generally, the lectin inhibited less the growth of fungi compared to the tested bacteria. PnL (100 μ g/mL in PDA medium) showed significant inhibition (24.4%) of mycelial growth against *Alternaria alternata* among all tested fungi (Table 5). On the other hand, the growth of *Botryodiplodia theobromae*, *Curvularia lunata* and *Colletotrichum corchori* (15.5-9.9%) was moderately inhibited by the lectin. However, the growth of *Fusarium equiseti* was least inhibited by the lectin. On the other hand, *Macrophomina phaseolina* was never inhibited by PnL, though the growth of all the six fungi was totally inhibited by nystatin (100 μ g/mL in PDA). A mannose/glucose-binding lectin from red cluster

pepper (*Capsicum frutescens*) inhibited the growth of two fungi as *Fusarium moniliforme* and *Aspergillus flavus* [41]. And another mannose-binding lectin from *Pisum sativum* seeds lectin had also inhibited *Fosarium oxysporum*, *Aspergillus flavus* and *Trichoderma viride* fungi [42]. Although the growth inhibition effect by PnL against fungi was significant but not strong, some other glucose-, galactose-, mannose- or fucose-binding lectins from plants had significant antifungal activity as same as PnL [43]. However, since it was not a common activity that lectins inhibit the growth of fungi, the anti-fungal activity by PnL was significant.

During the course of evolution, marine invertebrates have developed defense strategies against various pathogens, firstly bacteria living in the surrounding water and sediment [44].

TABLE IV
GROWTH OF INHIBITORY EFFECTS BY THE PURIFIED LECTIN
AGAINST GRAM-NEGATIVE BACTERIA

Name of bacteria	Diameter zone of inhibition in mm	
	Lectin (250 µg/disc)	Ampicillin* (20 µg/disc)
<i>Salmonella typhi</i>	6±1	20±1
<i>Salmonella paratyphi</i>	5±1	21±1
<i>Shigella dysenteriae</i>	4±1	18±1
<i>Shigella sonnei</i>	4±1	19±1
<i>Escherichia coli</i>	3±1	18±1
<i>Vibrio cholerae</i>	0	20±1
<i>Pseudomonas sp</i>	0	17±1

*Standard antibacterial antibiotic, Statistical analysis (RBD) at 1% level, organisms significant (F value 183.5), replica significant (F value 6.95).

TABLE V
ANTIFUNGAL ACTIVITY OF THE PURIFIED LECTIN AGAINST
PHYTOPATHOGENIC FUNGI

Name of bacteria	Percentage inhibition of fungal mycelial growth	
	Lectin (100 µg/mL)	Nystatin* (100 µg/mL)
<i>Alternaria alternata</i>	24.4±1	50.2±1
<i>Botryodiplodia theobromae</i>	15.5±1	52.2±1
<i>Curvularia lunata</i>	11.5±1	56.7±1
<i>Colletotrichum corchori</i>	9.9±1	44.5±1
<i>Fusarium equiseti</i>	8.7±1	38.6±1
<i>Macrophomina phaseolina</i>	0	55.5±1

*Standard antifungal antibiotic, growth measured-radial growth in cm.

Reactions against these living pathogens constitutes immune defense. Sessile invertebrates, such as corals, sponges and ascidians are well known to produce an astonishing variety of antimicrobial compounds [45], which help to control bacterial surface colonization [46]. By contrast only a limited number of polychaeta species have been investigated in this respect. The present study has shown that PnL possesses significant antibacterial and antifungal activity against some selected pathogenic microorganisms. Although earlier reports of the antimicrobial activities of lectins have been published, this is the first time to report on the antibacterial and antifungal activities of *P. nuntia* lectin.

IV. CONCLUSION

The present study have shown that PnL possess significant growth inhibition effects against some human and phytopathogens. *Perinereis nuntia* lectin (PnL) was screened for the first time for antimicrobial activities in the literature.

APPENDIX

Abbreviations used:

PnL: *Perinereis nuntia*; TBS: Tris-buffered saline; PBS: Phosphate buffered saline; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; GPC: Gel permeation chromatography; BCA: Bicinchoninic acid; NA: Nutrient agar; PDA: Potato dextrose agar.

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