Growth inhibitory effects of a GlcNAc/GalNAc-specific lectin from the marine demosponge *Halichondria okadai* on human pathogenic microorganisms

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Abstract

The aim of this study was to demonstrate the microbial usefulness of GlcNAc/GalNAc-specific lectin obtained from the marine demosponge, *Halichondria okadai* by affinity chromatography. For this purpose, we investigated the growth inhibitory effects of this lectin on bacterial strains. Through SDS-polyacrylamide gel electrophoresis, lectin was found to be a 18 kDa polypeptide. This lectin showed significant hemagglutinating activity against trypsinized human and rabbit erythrocytes and it was inhibited by N-acetyl glucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc). Lectin was screened for in vitro antibacterial activity against eleven human pathogenic bacteria and significantly inhibited the growth of gram-positive bacteria more than gram-negative bacteria. Antibacterial evaluation of standard antibodies, ampicillin was used as comparative study. *Bacillus megaterium* (13±1 mm) and *Staphylococcus aureus* (11±1 mm) were exhibited the highest zone of inhibition by the addition of the lectin (250 µg/disc). At the same time, lectin showed good growth inhibition against the gram-negative bacteria such as *Shigella sonnei* (10±1 mm), *Salmonella typhi* (08±1 mm) and *Vibrio cholerae* (07±1 mm). However, it did not inhibit the growth of gram-negative bacteria as *Escherichia coli* and *Pseudomonas* sp. These antibacterial results indicate that future findings of lectin applications obtained from marine demosponge may be of importance to clinical microbiology and could have possible applications in therapeutic.

Keywords: *Halichondria okadai*, demosponge, lectin, microorganisms, antibacterial activity.

Denizel Demospongia *Halichondria okadai* GlcNac/GalNAc-özgün lektinin insan patojenik mikroorganizmaları üzerindeki büyüme önleyici etkileri

Özet

Bu çalışmamın amacı denizel Demospongia *Halichondria okadai*’den elde edilen GlcNac/GalNAc’ye özgü lektinin mikrobiyal yararlarının afinite kromatografisi ile gösterilmesidir. Bunun için lektinin bakteri suşlarında büyümeyi engelleyici etkilerini araştırıldı. SDS-poliakrilamid jel elektroforez ve lektinin 18kDA polipeptit olduğu bulundu. Bu lektin tripsinize edilmiş insan ve tavan eritrositlerine karşı önemli derecede çökme aktivitesi gösterdi ve bu N-asetil gluokozamin (GlcNAc) ve N-asetil galaktozamin (GalNAc) ile engellendi. Lektin on bir insan patojenik bakterisine karşı antibakteriyel aktivitesi için test edildi ve belirgin olarak gram pozitif bakterilerin büyümelerini gram-negatif bakterilerle göre daha çok engelledi. Karşılaştırmalı çalışma olarak standart antibiyotik, amfisillin antibakteriyel ölçümdü kullanıldı. *Bacillus megaterium* (13±1 mm) ve *Staphylococcus aureus* (11±1 mm) lektin (250 µg/disk) eklenmesi ile en yüksek engellemenin dilimini
gösterdi. Aynı zamanda, lektin gram-negatif bakteri olan *Shigella sonnei* (10±1 mm), *Salmonella typhi* (08±1 mm) ve *Vibrio cholerae*’ye (07±1 mm) karşı iyi derecede büyüme baskı gösterdi. Ancak, *Escherichia coli* ve *Pseudomonas* türleri gibi gram-negatif bakterilerin büyümelerini baskılamadı. Bu antibakteriyel sonuçlar denizel Demospongia *Halichondria okadai*’larından elde edilen lektin uygulamaları ile ilgili gelecek bulguların klinik mikrobiyoloji için önemli olabileceğini ve terapide olası uygulamaları içrebileceğini işaret eder.

**Anahtar sözcükler:** *Halichondria okadai*, demosponge, lektin, mikroorganizma, antibakteriyel aktivite.

**Introduction**

Lectins are carbohydrate-binding proteins present in a diversity of organisms including humans, vertebrates and invertebrates, plants and fungi (Medeiros et al., 2010). They display a host of biological activities such as antitumor (Lam et al., 2009), antibacterial (Oliveira et al., 2008), anti fungal (Yan et al., 2009) and antiviral (Leung et al., 2008). Marine organisms are attractive for lectinologists as new origins and sources of unusual lectins. In recent years, a variety of lectins have been purified and characterized from marine invertebrates (Kawasar et al., 2009; Takahashi et al., 2008; Moura et al., 2006; Molchanova et al., 2005). Although in most cases physiological functions of invertebrate lectins are not completely clear, there are increasing data suggesting the involvement of these lectins in processes of differentiation and development of organisms as well as in elimination of foreign substances through binding to their carbohydrate structures (Kawsabata and Tsuda 2002; Espinosa et al., 2001).

Gram-positive bacteria are those that are stained blue or violet by Gram staining. This is a contrast to Gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counter stain (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 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 layer in gram-negative bacteria or remains exposed on the surface of gram-positive bacteria. Peptidoglycan is a polymer of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid units connected by short pentapeptides. The β-1,4-glycosidic bond of the *N*-acetylglucosamine-*N*-acetylmuramic acid peptide - glyc an backbone can be hydrolyzed by lysozyme (muramidase; mucopeptide *N*-acetyl muramoylhydro-lase), a ubiquitous enzyme involved in innate immune reaction of numerous animal species (Ito et al., 1999).

Sponges, the evolutionarily oldest metazoan phylum (*Porifera*), share one common ancestor with the other metazoan phylum, the *Urmetazoa* (Muller et al., 2001). Most sponges are marine sessile filter feeders; some species daily filter tons of water containing millions of bacteria or viruses through their bodies (Reiswig, 1974). Due to their efficient chemical and biological defense systems, sponges have survived not only adverse climatic periods but also biotic and anthropogenic threats (Muller et al., 2000). Two strategies are used by sponges to eliminate attacking (infectious) bacteria: first, chemical strategies via the production of secondary metabolites (Proksch, 1994) and second, humoral and cellular defense mechanisms (Muller et al., 1999). Sponges are the most primitive invertebrates and their water-extractable lectins have been localized within the spherulous cells, which are assumed to participate in the formation of the sponging fibers (Bretting et al., 1983). Interestingly, it has been theorized that some marine invertebrate lectins mediate the interaction between symbiont and host (Vasta, 1992). A 27 kDa lectin was purified from demosponge *Suberites domuncula* and displayed antibacterial activity against the gram-positive bacteria as *Staphylococcus aureus* and the gram-negative bacteria, *Escherichia coli* (Schorder et al., 2003). CvL, a D-galactose-binding lectin from marine sponge *Cliona varians* exhibited good antibacterial effect on gram-positive bacteria,
such as Bacillus subtilis and Staphylococcus aureus but it did not affect gram-negative bacteria, such as Escherichia coli and Pseudomonas aeruginosa (Moura et al., 2006). Haug et al., (2004) showed that marine invertebrates possess antipathogen factors in several tissues. In addition, Manila clam, Ruditapes philippinarum lectin significantly inhibited the growth of inhibition of Vibrio tubiashii bacteria (Takahashi et al., 2008).

Marine sponge, Halichondria okadai belong to the class of Demospongia is a toxic black sponge and potent toxins, okadaic acid and halichondrins (Tachibana et al., 1981) were isolated from this sponge. Also three lectins, HOL-I, HOL-II (Kawagishi et al., 1994) and HOL-30 (Kawar et al., 2008) have been purified from this same species using affinity chromatography. The purpose of this study was taken to evaluate the in vitro growth of inhibition of this lectin against eleven human pathogenic microorganisms as first time.

Materials and methods

Chemicals reagents

Chitto-oligo-agarose (polymer of GlcNAc) 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. Bicinchoninic acid (BCA) kit was purchased from Pierce Co. Ltd., USA. N-acetyl glucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc) were purchased from Wako Pure Chemical Ind. Ltd., Tokyo, Japan. Agar, dextrose, peptone, beef extract were purchased from Merck Ltd., India and BDH Ltd., Bangladesh.

Samples

Marine demosponges, Halichondria okadai (800 g wet weight) were collected in the tidal zone at the Zushi coast, Kanagawa Prefecture, Japan and were stored at -80°C or used after collection according to the situation.

Affinity purification of H. okadai lectin

The marine sponges (200 g) were homogenized in a commercial blender (Waring, USA) with 10 volumes (w/v) of Tris-buffered saline (TBS) (10 mM Tris(hydroxymethyl)aminomethane-HCl, pH 7.4, containing 150 mM NaCl) containing 10 mM of a protease inhibitor mixture. The homogenates were roughly filtered via a filter bag in a sink to remove debris. The filtrates 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. The supernatant was centrifuged again at 27,500 g for 1 h at 4°C for two times and was applied to a chitto-oligo-agarose affinity column that was fitted with a Sephadex G-75 pre-column. After application of the extracts, the column was washed extensively with TBS. The lectin was eluted with 100 mM N-acetyl glucosamine in TBS and each 1 ml of elution was collected in tubes with a fraction collector. Each chromatography step during washing and elution was monitored using a UV monitor by the measurement of the absorbance at 280 nm. The eluted fractions as identified by UV spectrophotometer was combined, and dialyzed against 1,000 times volumes of TBS to remove sugar from fractions.

Hemagglutinating activity

The hemagglutinating activity was performed using 1% (w/v) trypsinized and 0.25% glutaraldehyde-fixed rabbit and human erythrocytes as described previously (Matsui 1984). Erythrocytes were suspended at a concentration of 1% (w/v) in TBS. In the general assay, 20 µl each of TBS, TBS containing 1% Triton X-100, and erythrocytes were added to 20 µl 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 µl 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.

Molecular weight determination by SDS-PAGE

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 (Laemmli 1970). 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.

**Protein determination**

Protein concentrations were determined using BCA protein assay kit (Smith et al., 1985; Wiechelman et al., 1988) with bovine serum albumin as the standard by measuring absorbance at 562 nm with a spectrophotometer ND-1000 (Nano Drop Tech. Inc., USA).

**Microorganisms**

The bacterial 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).

**Culture and medium**

Standard NA (Nutrient Agar) 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 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.

**Growth inhibition assay**

The in vitro sensitivity of the bacteria to the test purified lectin was done by disc diffusion method (Bauer et al., 1996). 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 petri dishes 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 20 µl (250 µg/disc) from 5% phosphate buffered saline (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. N-acetyl glucosamine (GlcNAc) was used as negative control. All the results were compared with the standard antibacterial antibiotic ampicillin [20 μg/disc, BEXIMCO Pharma Bangladesh Ltd.].

Statistical analysis

![Graph](image_url)

**Figure 1.** Affinity purification of H. okadaii lectin. Crude extract of H. okadaii was applied to a chitto-oligo-agarose column equilibrated with TBS. The column was washed with TBS and eluted with TBS containing 100 mM N-acetyl glucosamine (GlcNAc) (arrow). The column bound fractions shown by the bar were collected and designated as purified lectin after dialysis against TBS.

**Table 1.** Purification of GlcNAc/GalNAc-binding lectin from the demosponge H. okadai

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Titer (HU)</th>
<th>Volume (ml)</th>
<th>Total activity a</th>
<th>Protein conc. (mg ml⁻¹)</th>
<th>Specific activity b</th>
<th>Purification ratio (fold)</th>
<th>Recovery of activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>256</td>
<td>200</td>
<td>51,200</td>
<td>2.51</td>
<td>0.51</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Affinity purification</td>
<td>2048</td>
<td>8.5</td>
<td>17,408</td>
<td>0.81</td>
<td>297</td>
<td>582</td>
<td>34</td>
</tr>
</tbody>
</table>

aTotal activity is shown by Titer × volume.
bSpecific activity was shown by titer/mg of protein.

Statistical analyses were done by using Student’s t-test with organism’s significant F value 183.5 and replica significant F value 6.95.

**Results and Discussion**

Bioactive carbohydrates, proteins, peptides and secondary metabolites have been isolated from various marine invertebrate classes, including the
bio-prospection of Porifera, which may be of tremendous potential benefit for humans. Among these bioactive molecules, lectins are proteins involved in the non-immune responses in invertebrate marines and their effects on the antibiosis of pathogenic organisms have been reported (Sharon 2007; Gabius et al., 2002). *H. okadai* lectin was purified from the marine demosponge by chito-oligo-agarose column chromatography via elution with 100 mM N-acetyl glucosamine (GlcNAc) containing TBS (Figure 1).

About seven milligrams of *H. okadai* lectin was successfully purified from 200 g of marine sponge (wet mass) and it was concentrated 582 times by affinity purification (Table 1).

It was shown to be a single polypeptide with molecular mass 18 kDa under non-reducing (NR) and reducing (R) conditions by SDS-PAGE, respectively (Figure 2). The purified lectin was agglutinated with trypsinized and glutaraldehyde-fixed human and rabbit erythrocytes.

![SDS-PAGE gel](image)

**Figure 2.** SDS-polyacrylamide gel electrophoresis of *H. okadai* lectin. Purified lectin (L), (10 µg) was subjected to 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); and lysozyme (14 kDa). ‘*’ indicates purified lectin.

<table>
<thead>
<tr>
<th>Erythrocytes source*</th>
<th>Titer (HU)</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td></td>
</tr>
<tr>
<td>Type A</td>
<td>2048</td>
</tr>
<tr>
<td>Type B</td>
<td>1024</td>
</tr>
<tr>
<td>Type O</td>
<td>1024</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2048</td>
</tr>
</tbody>
</table>

*Trypsinized and glutaraldehyde fixed erythrocytes were used.
in the absence of any divalent cations (Table 2). The hemagglutinating activity of the lectin was specifically cancelled by the co-presence of N-acetyl glucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc).

The purified lectin had so similar physicochemical properties with another tetrameric 21 kDa lectin (Kawagishi et al., 1994) purified from same species on the affinity to N-acetyl sugar and the independence from divalent cations for its activity. A dimeric 30 kDa D-galactoside specific lectin was also purified from this same species (Kawsar et al., 2008). This result indicated that marine demosponge of H. okadai contain multiple lectins. Lectin can specifically recognize the N-acetyl group of the terminal GlcNAc residue as methyl α-D-glucosamine (Me αGlcNAc), methyl β-D-glucosamine (Me β GlcNAc), methyl α-D-galactosamine (Me αGalNAc), methyl β-D-galactosamine (Me βGalNAc), D-galactose (D-Gal) and lactose (Galβ1-4Glc) was not recognized by the lectin. From these results, H. okadai lectin has been characterized as an N-acetyl glucosamine or N-acetyl galactosamine lectin as same as ACL-I purified from marine sponge of Axinella corrugate (Dresch et al., 2008) and DTL purified from ascidian, Didemnum ternatanum (Molchanova et al., 2005). The lectins interact with the carbohydrate moieties in different ways: (a) interacts as typical C-type lectins via a Ca$^{2+}$ ion, (b) interacts with specific single, terminal sugars, without the help of a metal cation; (c) involved in more intimate interactions, with multiple carbohydrate rings and no metal ion; (d) interaction mode has not yet been elucidated (Botos and Wlodawer 2005). It has also been reported that several lectins isolated from various marine invertebrates which can agglutinate with various bacterial pathogens and fungi (Tsumi et al., 2004; Tateno et al., 2002).

H. okadai lectin was subjected to screening for in vitro antibacterial inhibition growth against eleven pathogenic bacteria and compared to that of antibacterial antibiotic, ampicillin. The results of the sensitivity test are presented in Table 3 and 4. H. okadai lectin (250 µg/disc) exhibited a strong antibacterial activity on the gram-positive bacteria, as *Bacillus megaterium* and *Staphylococcus aureus*. The diameter of zone inhibition by the addition of lectin was significant effective for *Bacillus megeterium* and *Staphylococcus aureus* to be 13 and 11 mm, respectively (Table 3). This result is very similar with 28 kDa D-galactose-binding from marine sponge Cliona varians which is inhibited the growth of *Staphylococcus aureus* and *Bacillus* species bacteria (Moura et al., 2006). However, the lectin has inhibited less effect for *Bacillus cereus* and *Bacillus subtilis*. On the other hand, lectin showed good antibacterial activity against

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Lectin (250 µg/disc)</th>
<th>Ampicillin* (20 µg/disc)</th>
</tr>
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<tbody>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>13±1</td>
<td>19±1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11±1</td>
<td>21±1</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>4±1</td>
<td>18±1</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>4±1</td>
<td>19±1</td>
</tr>
</tbody>
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Note: *Standard antibacterial antibiotic. Statistical analysis (RBD) at 1% level,*
organisms significant (F value 183.5), replica significant (F value 6.95).
gram-negative bacteria such as Shigella sonnei, Salmonella typhi and Vibrio cholerae but it did not inhibit the growth of other gram-negative such as Escherichia coli and Pseudomonas sp (Table 4), though the control antibiotic, ampicillin inhibited the growth against all gram-negative bacteria. Amongst the gram-positive and gram-negative bacteria, gram-positive bacteria were more effective to the lectin as compared to gram-negative bacteria. This result suggest that the surface-exposed carbohydrates of bacteria were different even if they are belong same genus such as Bacillus. The glycomics approach to determine the structure of surface glycans of bacteria will provide us with more useful information to prevent the diseases using lectins. Some lectins seem to be useful for

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Diameter of zone of inhibition in millimeter</th>
</tr>
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<tbody>
<tr>
<td>Lectin (250 µg/disc)</td>
<td>Ampicillin* (20 µg/disc)</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>10±1</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>8±1</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>7±1</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>4±1</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>4±1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: *Standard antibacterial antibiotic. Statistical analysis (RBD) at 1% level.

organisms significant (F value 183.5), replica significant (F value 6.95).

identification of pathogenic bacteria based on the specific binding moieties of lectins to the characteristic glycans on cell wall of bacteria (Munoz-Crego et al., 1999).

Our antibacterial results pattern of differentiation was observed among lectins from diverse origins because almost all microorganisms express surface-exposed carbohydrates as a potential lectin-reactive specific site. This property is exemplified in the invertebrate marine Tachypleus tridentatus, where several lectins, denominated tachylectins 1, 2, 3 and 4, were purified from their hemolymph. Tachylectin 1 was able to inhibit the growth of gram-negative bacteria (Saito et al., 1995). Tachylectin 2 had a binding specificity for N-acetyl glucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc) and promoted the agglutination of certain strains of gram-positive Staphylococcus (Kawabata and Iwanaga 1999; Okino et al., 1995) which is much fitted with our result. Tachylectin 3 and 4 recognized the lipopolysaccharide (LPS) of several gram-negative bacteria (Inamori et al., 1999; Saito et al., 1997). The binding of H. okadai lectin to N-acetyl group sites in bacterial surface could explain deterrent effects found in agglutinating tests with gram-positive bacteria. The property of these lectins to select and complex with microbial glyco-conjugates has made it possible to employ the proteins as probes and sorbents for whole cells, mutants and numerous cellular constituents and metabolites. Recently, a β-galactoside binding pearl shell lectin purified from marine bivalve, Pteria penguin (Naganuma et al., 2006) had shown the similar antibacterial activity with purified lectin, as
it effectively inhibited the growth against both gram-positive and gram-negative bacteria. Also rhamnose-binding steelhead trout (Oncorhynchus mykiss) eggs lectin inhibited the growth of gram-positive and gram-negative bacteria by recognizing lipopolysaccharide or lipoteichoic acid (Tateno et al., 2002) as same as H. okadai lectin. H. okadai contain a large quantity of GlcNAc/GalNAc-binding lectin but its physiological function is unknown yet. The presence of the lectin in the demosponge of H. okadai led us to consider its possible biological involvement in the defense mechanisms of the species. The wide distribution of lectin with antimicrobial property in the animal kingdom indicates that this lectin has been well conserved during evolution, which is understandable because animals cannot survive unless they can eliminate invading bacteria. Lectins isolated from marine sponges have turned into a fast growing field in life sciences due to their extensive physiological, biological and pharmacological uses. Since the lectin can be purified in big amounts from the demosponge H. okadai, and for inhibition of growth against some pathogens it will be expectable to be a potential drug discovery targets for therapeutic agents or diagnostic probes.

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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.

N-acetylglucosamine (GlcNAc) was used as negative control. All the results were compared with the standard antibacterial antibiotic ampicillin [20 µg/disc, BEXIMCO Pharma Bangladesh Ltd.].

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Bioactive carbohydrates, proteins, peptides and secondary metabolites have been isolated from various marine invertebrate classes, including the bio-prospection of Porifera, which may be of tremendous potential benefit for humans. Among these bioactive molecules, lectins are proteins involved in the non-immune responses in invertebrate marines and their effects on the antibiosis of pathogenic organisms have been reported (Sharon 2007; Gabius et al., 2002).

H. okadai lectin was purified from the marine demosponge by chitto-oligo-agarose column chromatography via elution with 100 mM N-acetylglucosamine (GlcNAc) containing TBS (Figure 1). About seven milligrams of H. okadai lectin was successfully purified from 200 g of marine sponge (wet mass) and it was concentrated 582 times by affinity purification (Table 1).

It was shown to be a single polypeptide with molecular mass 18 kDa under non-reducing (NR) and reducing (R) conditions by SDS-PAGE, respectively (Figure 2). The purified lectin was agglutinated with trypsinized and glutaraldehyde-fixed human and rabbit erythrocytes in the absence of any divalent cations (Table 2). The hemagglutinating activity of the lectin was specifically cancelled by the co-presence of N-acetyl glucosamine (GlcNAc) and N-acetyl galactosamine (GalNAc).

The purified lectin had so similar physicochemical properties with another tetrameric 21 kDa lectin (Kawagishi et al., 1994) purified from same species on the affinity to N-acetyl sugar and the independence from divalent cations for its activity. A dimeric 30 kDa D-galactoside specific lectin was also purified from this same species (Kawsar et al., 2008). This result indicated that marine demosponge of H. okadai contain multiple lectins. Lectin can specifically recognize the N-acetyl group of the terminal GlcNAc residue as methyl α-D-glucosamine (Me αGlcNAc), methyl β-D-glucosamine (Me β GlcNAc), methyl α-D-galactosamine (Me αGalNAc), methyl β-D-galactosamine (Me βGalNAc). D-galactose (D-Gal) and lactose (Galβ1-4Glc) was not recognized by the lectin. From these results, H. okadai lectin has been characterized as an N-acetyl glucosamine or N-acetyl galactosamine lectin as same as ACL-I purified from marine sponge of Axinella corrugate (Dresch et al., 2008) and DTL purified from ascidian, Didemnum ternatanum (Molchanova et al., 2005). The lectins interact with the carbohydrate moieties in different ways: (a) interacts as typical C-type lectins via a Ca\(^+2\) ion, (b) interacts with specific single, terminal sugars, without the help of a metal cation; (c) involved in more intimate interactions, with multiple carbohydrate rings and no metal ion; (d) interaction mode has not yet been elucidated (Botos and Wlodawer 2005). It has also been reported that several lectins isolated from various marine invertebrates which can agglutinate with various bacterial pathogens and fungi (Tsumi et al., 2004; Tateno et al., 2002).

H. okadai lectin was subjected to screening for in vitro antibacterial inhibition growth against eleven pathogenic bacteria and compared to that of antibacterial antibiotic, ampicilin. The results of the sensitivity test are presented in Table 3 and 4. H. okadai lectin (250 µg/disc) exhibited a strong antibacterial activity on the gram-positive bacteria, as Bacillus megaterium and Staphylococcus aureus. The diameter of zone inhibition by the addition of lectin was significant effective for Bacillus megeterium and Staphylococcus aureus to be 13 and 11 mm, respectively (Table 3). This result is very similar with 28 kDa D-galactose-binding from marine sponge Cliona varians which is inhibited the growth of Staphylococcus aureus and Bacillus species bacteria (Moura et al., 2006). However, the lectin has inhibited less effect for Bacillus cereus and Bacillus subtilis. On the other hand, lectin showed good antibacterial activity against...