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ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF A NOVEL CHITIN-BINDING LECTIN ISOLATED FROM 'DESHI' POTATO

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ABSTRACT

After subjecting the 'Deshi' potato (Solanum tuberosum L.) to anion-exchange and affinity chromategraphies with a chitin column, a novel lectin that binds to chitin was isolated. The lectin's molecular mass was determined to be 20,000 Daltons using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This molecular mass was about half that of lectins obtained from other potatoes that bind chitin. Listeria monocytogenes and Escherichia coli, Salmonella enteritidis, and Shigella boydii were among the Gram-positive and Gram-negative harmful bacteria that the lectin was shown to kill. Rhizopus spp., Penicillium spp., and Aspergillus niger were also shown to be susceptible to its antifungal effects.

Keywords: Antibacterial, Chitin, Lectin, Solanum tuberosum, Antifungal

I. INTRODUCTION

A lectin's ability to attach to chitin—a long-chain polysaccharide made up of N-acetylglucosamine units—is its defining feature. Because of their binding capabilities, CBLs may disrupt the structural integrity of exoskeletons in insects and fungal cell walls. As a possible plant defense mechanism, CBLs' biological functions go beyond simple binding; they may also display antibacterial characteristics, preventing the development of pathogens. The structural characteristics and carbohydrate-binding specificity of CBLs are the main criteria for their classification. Because of their possible uses as natural antibacterial agents and in agriculture and medicine, they are quite intriguing. Research on CBLs derived from different plants may provide light on their distinct characteristics, allowing us to increase the variety of biopesticides and medicinal agents at our disposal. In particular, the potato chitin-binding lectin (Solanum tuberosum) is making waves in plant pathology and biotechnology because to its unique antibacterial and antifungal properties. Lectins are proteins that bind carbohydrates and display a broad variety of biological functions. The exoskeletons of insects and fungal cell walls both include chitin, which lectins designed to bind to chitin have a strong preference for. These lectins are essential parts of the plant's defense system because of their binding capacity, which gives them a strong antifungal and insecticidal effect. In potatoes, the chitin-binding lectin has been shown to be very effective in

destroying harmful organisms, protecting the plant from diseases and ensuring its overall health.

Native to the Indian subcontinent, the 'Deshi' potato (Solanum tuberosum L.) is a popular potato cultivar. The 'Deshi' potato variety has the potential to produce novel biochemical substances, such as lectins, due to its adaptation to local environmental circumstances, in contrast to the commercial varieties that have been extensively investigated. A variety of lectins with different biological activity are produced by potatoes. The 'Deshi' variety may have different characteristics as a result of its local adaptability and specific growing circumstances, however it has received less research. There is a chance to find new antimicrobial drugs with interesting features by isolating chitin-binding lectins from this kind.

The capacity of potato chitin-binding lectin to attach to the peptidoglycan layer in bacteria and the chitin in fungus cell walls is responsible for its antibacterial and antifungal actions. The pathogens' structural integrity is disrupted by this binding, which causes cell lysis and death. Fungal cell wall chitin binding by lectin inhibits cell wall formation and repair, leaving the cell more vulnerable to external stressors and ultimately leading to cell death. Bacterial cell wall formation is also interrupted by interactions with the peptidoglycan layer, which compromises cell integrity and ultimately causes cell death.

Similarly remarkable are the antimicrobial capabilities of the chitin-binding lectin found in potatoes. The bacterial infections caused by Pseudomonas syringae and Erwinia carotovora, as well as other pathogens, include soft rot in potatoes, but this lectin has the ability to prevent their development, according to research. Bacterial cell death occurs when the lectin binds to bacterial cell walls, causing them to rupture and release their contents. This antimicrobial effect promotes eco-friendly farming techniques by protecting potato plants from bacterial infections and decreasing the need for chemical bactericides.

The revelation that the chitin-binding lectin from potatoes has antibacterial and antifungal properties is a huge step forward for plant biotechnology. Researchers can create novel biocontrol tactics that work and are sustainable by using plants' innate defensive systems. An attractive alternative for conventional pesticides, which are linked to pollution and the rise of resistant disease strains, is the use of chitin-binding lectins for crop protection. The chitin-binding lectin from potatoes has uses beyond only farming. Because of its antibacterial characteristics, this lectin has great promise for use in the pharmaceutical and medical industries. It has many potential uses, such as a natural preservative in food and cosmetics and in the creation of novel antimicrobial medications. The significance of chitin-binding lectins as multifunctional proteins with broad-spectrum uses is highlighted by their adaptability. Lastly, Solanum tuberosum, a new lectin that binds chitin, is an effective weapon against plant diseases. Its strong antibacterial capabilities stem from its one-of-a-kind capacity to attach to chitin and compromise the structural integrity of bacterial and fungal cell walls.

II. REVIEW OF LITERATURE

Shi, Xiaozai et al., (2020) Since chitin is unique to fungus and not present in animals or plants, chitin synthase (CHS) might be a target for environmentally friendly fungicides. As CHS inhibitors, 35 maleimide compounds were produced and described in this work. In vitro, antifungal and CHS inhibitory activities varied among the tested drugs. Specifically, compound 20 had a stronger inhibitory impact than the control polyoxin B (IC50 = 0.19 mM) on CHS, with a half-inhibitory concentration (IC50) value of 0.12 mM. Simultaneously, this molecule exhibited promising antifungal action and has promise for further research.

Feng, Yun et al., (2018) There are two chitin-binding modules in the potato lectin, and each of these modules has two hevein domains. In this work, a straightforward, efficient, and time-saving method for the small-scale purification of potato lectin was established, using the thermotolerance of the hevein polypeptide. After the ammonium sulfate precipitation and heating treatment, the process only requires one anion exchange chromatographic step. The potato lectin, a glycoprotein with a molecular mass of around 60 kDa, was isolated and homogeneously purified using this approach. It exhibited 9513.3 u/mg of specific hemagglutination (HA) activity with a yield of 76.8%. Electrophoresis using SDS-PAGE and reverse-phase HPLC both verified the homogeneity. With a 100% Confidence Interval, the purified lectin was determined to be similar to hevein domains in potato lectin utilizing MS-based peptide sequencing (MALDI-TOF/TOF). In addition to the periodic acid-Schiff staining and ferric-orcinol test for pentose, the purified lectin was further validated as a chitin-binding lectin by inhibiting its HA activity with chitosan oligomers. The heat tolerance of the purified potato lectin was an important factor in the removal of over 96% of the total proteins from the crude extract, which was further enhanced by a brief precipitation process using ammonium sulfate. So, using a DEAE-methyl polyacrylate column, the lectin was readily separated from the other remnant proteins.

Iordache, Florin et al., (2015) One of the most pressing issues facing modern medicine, antibiotic resistance has emerged as a key worry for the twenty-first century. The rapid dissemination of newly-evolved microbial resistance mechanisms has increased the prevalence of nosocomial infections and cast doubt on the efficacy of current treatments for a wide range of infectious disorders. Lectins have demonstrated to mediate a wide variety of biological processes, including cytotoxicity, complement activation, cell-to-cell and host-pathogen communications, innate immune response, cell-to-cell signaling, and their usage as hemaglutinine and in the identification of complex carbohydrates and glycoconjugates in immunology and glycobiology. The antiparasitic and antibacterial properties of lectins have piqued a lot of interest in their study and prospective medical and agricultural uses recently. By outlining lectins' function in host-pathogen interaction and their cytotoxic effects on microbes and parasites, this review zeroes in on the most current evidence on lectins' antimicrobial and antiparasitic capabilities. An alternative to antibiotics for illnesses caused by parasites and microbes that are resistant

to these drugs might be found via the discovery and characterization of novel lectins that have antimicrobial activity.

Yan, Juan et al., (2015) Because of their complexity, fungi are able to cause a wide variety of plant diseases, which results in annual crop losses. To combat these fungus, plants have evolved a number of defense mechanisms, including as the synthesis of antifungal proteins and peptides and low-molecular-weight secondary compounds. This study will provide a concise overview of many families of plant antifungal proteins (AFPs), including as defensins, lectins, and others. Furthermore, we shall also examine how AFPs are used in farming.

Zarei, Mandana et al., (2011) Chitinases are enzymes that are capable of digesting chitin, which is a primary component of the cell wall of many phytopathogens, including fungus. Chitinases are found in many different types of plants. During the subsequent experiment, a native Serratia marcescens B4A strain was used to describe a new chitinase that exhibited antifungal activity. An enzyme that had been partially purified had a molecular mass that seemed to be 54 kDa. It stated that the optimal activity was pH 5 at 45 degrees Celsius. Enzyme was stable at a temperature of 55 degrees Celsius for twenty minutes and at a pH range of three to nine for ninety minutes at a temperature of twenty-five degrees Celsius. It is possible that the structure of enzymes was altered when the temperature was elevated to sixty degrees Celsius, which resulted in a decrease in the activity of chitinase. In addition to this, the Km and Vmax values for chitin were 8.3 mg/ml and 2.4 mmol/min, respectively. In addition, it was discovered that the activity of chitinase was stimulated by the influence of certain cations and chemical compounds. In addition, the activity of the enzyme was not inhibited by iodoacetamide or idoacetic acid, which suggests that cysteine residues are not a component of the catalytic site of chitinase. In conclusion, chitinase activity was further monitored by scanning electronic microscopy data, which revealed that treatment with chitinase resulted in gradual alterations in the porosity of chitin. This enzyme demonstrated antifungal activity against Rhizoctonia solani, Bipolaris sp., Alternaria raphani, and Alternaria brassicicola, so indicating a prospective application for the industry that has the ability to carry out exploitable importance. Chitin produced by fungi has a number of distinctive characteristics, particularly with regard to its chemical structure. It is necessary that the subsite structure in the enzyme binding cleft be the cause of the difference in chitinolytic capacity. Given these information, it is clear that the enzyme did not exhibit considerable antifungal efficacy against other types of fungi.

Kabir, Syed Rashel et al., (2010). Using affinity chromatography on a chitin column, chitinases (identified as SPCs) were recovered from the 'Shilbilati' potato prototype, which is grown in Bangladesh. SPCs exhibited toxicity against brine shrimp nauplii with an LC50 value of 20 µg/mL and agglutinated rat erythrocytes at a minimum concentration of 7 µg/mL. Out of the twelve bacterial strains that were investigated, seven of them were agglutinated by the chitinases. At protein concentrations of

1.2, 2.5, and 5.0 µg/mL, respectively, Pseudomonas aeruginosa, Bacillus subtilis, and Salmonella typhi were shown to be the most susceptible to SPCs and agglutinated. Staphylococcus aureus, Bacillus subtilis, and Salmonella typhi were among the harmful bacteria that SPCs inhibited in antibacterial testing. The disc diffusion technique was used to test antifungal activity. The test looked at two fungal genera (Penicillium and Mucor sp.) and five different species of fungus (Candida albicans, Aspergillus niger, Fusarium vasinfectum, Aspergillus fumigatus, and Aspergillus flavus). Candida albicans, Fusarium vasinfectum, and Penicillium sp. were all inhibited by SPCs.

Khoushab, Feisal & Yamabhai, Montarop. (2010) Almost everyone now agrees, even after two centuries, that chitin is a crucial biopolymer for several reasons. The biomedical uses of chitin have been the subject of a great deal of research. This review will cover a diverse variety of topics related to chitin, including its origins, structure, biosynthesis, chitinolytic enzyme, chitin binding protein, genetic engineering method for chitin production, chitin and evolution, and its many bio- and nanotechnological uses.

III. MATERIALS AND METHODS

We bought some potato tubers (Solanum tuberosum L. cv. 'Deshi') at the local market and kept them in the fridge at 4 degrees Celsius.

Chemical Industries Ltd. supplied the chitin and DEAE-cellulose. A business in India supplied the automated microtiter plate reader. This investigation only made use of analytical-grade chemicals and reagents.

Purification of lectin

We cut and blended 400 g of potatoes till smooth after removing the skins. The addition of 10 mM Tris-HCl (pH 8.2) and 50 mM NaCl further enhanced the mixture's smoothness. The fluid above the solid was retrieved following fifteen minutes of spinning at 20,000 × g and then dialyzed against the same buffer at 4°C for a duration of six hours. The hemagglutination test was conducted using plates that had 96 wells and a U-bottom. In a solution of 150 mM NaCl, 10 mM Tris-HCl, and pH 8.2 (TBS), the crude potato extract was diluted twice. Then, 20 l of a 2% culture of mouse erythrocytes (with TBS; v/v) was mixed with it. After that, the plate was given half an hour to incubate at ambient temperature. By watching for the formation of a sheet (agglutination-positive) or a dot (agglutination-negative), the hemagglutination titer was ascertained. A DEAE-cellulose column (2 × 25 cm) was used to load the unprocessed supernatant, which was eluted using a linear gradient of 0 to 400 mM NaCl containing 10 mM Tris-HCl (pH 8.2).

The 2.5 mL hemagglutination-active fractions were loaded onto a 2 × 25 cm chitin column that had been equilibrated with TBS and was then eluted with 0.5 M acetic acid. An aliquot of 1 M Tris-HCl buffer (pH 8.2) was added to each tube to neutralize the pH of the eluted fractions, and the tubes were

dialyzed overnight. Following prior instructions, we used SDS-PAGE on a 15% separating gel in a reducing environment to measure the molecular mass and hemagglutination activity of the purified lectin.

Antibacterial assay

A mixture of Salmonella enteritidis ATCC 13076, Escherichia coli O157:H18, Listeria monocytogenes ATCC 35152, and Shigella boydii ATCC 9905 was grown overnight at 37ºC in a liquid nutritional medium. Every bacterial strain was first spun at 4000 × g for three minutes, and then sorted into pellets. After that, they were washed with 20 mM Tris-HCl buffer saline (pH 7.8) and redissolved in the same buffer, but this time with a turbidity of 1.0 at optical density (OD) at 640 nm ("OD640"). In order to determine the purified lectin's antibacterial activity, the agar disc diffusion method was used in conjunction with 30 mL of nutritional agar in sterile petri plates.

In a separate step, harmful bacteria were spread out on plates. Then, ordinary paper discs were layered on top of the agar. After coating the discs with 50 µg of potato lectin in 100 µl of solution, the bacterial cells were allowed to proliferate at 30ºC for 12 hours. A transparent ring encircling the paper discs demonstrated their antibacterial action.

Antifungal assay

The lectin's antifungal activity was tested using the agar disc diffusion technique against Rhizopus spp., Penicilium spp., and Aspergillus niger, three different fungal strains. Thirty milliliters of potato dextrose agar was placed on sterile petri plates. Mycelia from several fungi were cultured on solid potato dextrose agar with sterile filter paper discs placed on top. One hundred microliters (containing fifty grams) of pure lectin was used to soak the discs. The petri dishes were kept at 30°C until mycelial growth was seen. Around the lectin-containing discs, translucent zones resembling crescents emerged.

Statistical analysis

The mean plus or minus the standard error (SE) was used to represent the experimental findings. A two-tailed Student's t-test was used to assess differences in means, and statistical significance was defined as P values less than 0.05.

IV. RESULTS AND DISCUSSION

Purification of lectin

There was a substantial hemagglutinating action against mouse erythrocytes in the crude supernatant obtained from the Deshi potato variety. As is typical of other potato lectins4, the addition of chitotriose selectively decreased the activity, whereas GlcNAc had no effect in the microtiter plate experiment (data not shown). The DEAE-cellulose column was subjected to anion-exchange chromatography of the supernatant, and four peaks were eluted from the column using a linear gradient of NaCl concentration (0-400 mM) (Fig. 1). Because chitin has a considerable hemagglutinating activity, the

first peak's fractions were blended and put onto it.

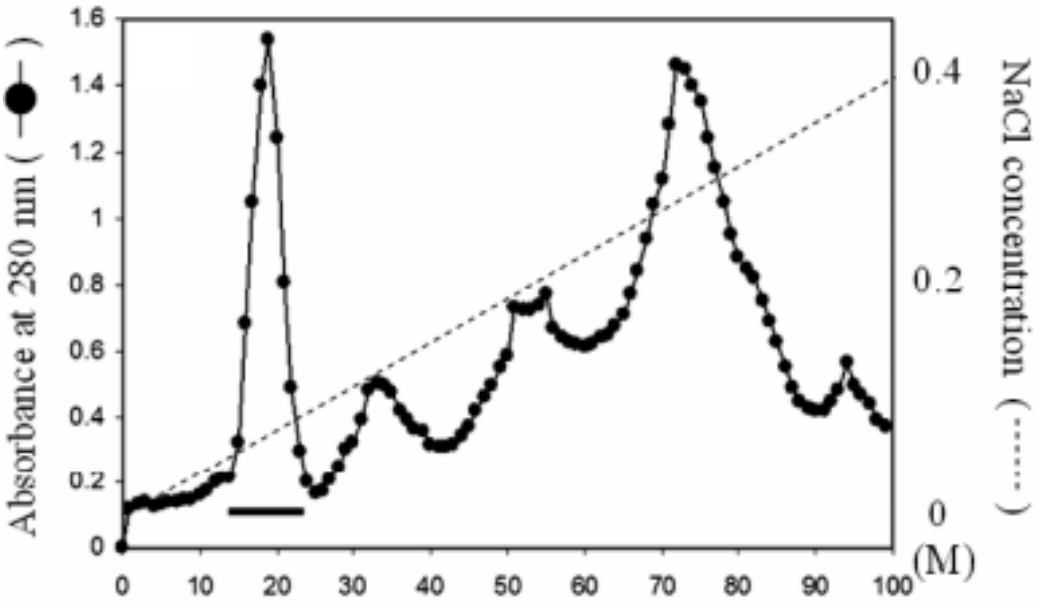

Figure 1: Purification of StL-20: DEAE-Cellulose Chromatography

After extensively washing the chitin column with TBS, a concentration of 500 mM acetic acid was employed to extract a solitary peak (Fig. 2). After neutralizing the eluted fractions with a portion of 1 M Tris-HCl (pH 8.2), we subjected them to dialysis against TBS. Figure 3 demonstrates that when exposed to reducing conditions, the 20 kDa chitin-binding lectin of S. tuberosum L. cv. Deshi (StL-20) was divided into a noticeable 20 kDa band and two smaller bands, measuring 22 and 17 kDa, respectively, using SDS-PAGE. Table 1 demonstrates that from a total of 400 grams of Deshi potatoes, about 16 milligrams of StL-20 were isolated, resulting in a lectin recovery rate of 33% (weight/weight).

Table 1 Purification of Lectin

Purification steps	**Titer (HU)**	**Volume (mL)**	**Total activity**	**Protein conc. (mg/mL)**	**Specific activity**	**Purification ratio (fold)**	**Recovery of activity (%)**
Crude extract	256	400	76,800	2.5	0.36	1	100
Ion-exchange chromatography	512	66	33,792	0.8	9.68	29	44
Affinity chromatography	1024	25	25,600	0.65	16.28	48	33

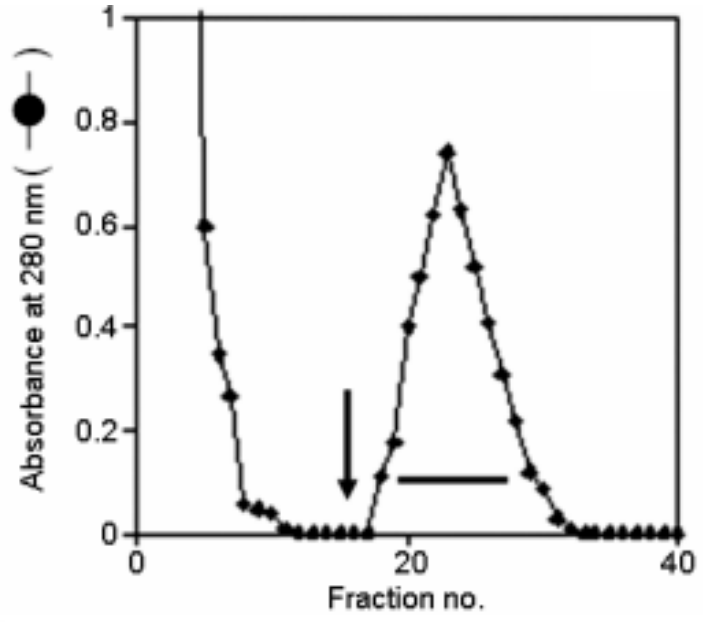

Figure 2: Purification of StL-20: Chitin Column Elution

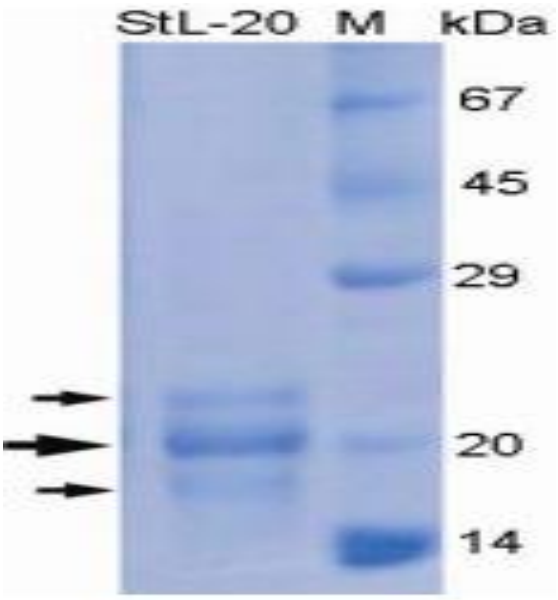

Figure 3: Molecular mass by SDS-PAGE

Antibacterial activity

StL-20 exhibited strong antibacterial action against E. coli O157:H18, L. monocytogenes ATCC 35152, S. enteritidis ATCC 13076, and S. boydii ATCC 9905, as seen in Figure 4. Figure 5, column 3 demonstrates that the lectin exhibited greater sensitivity towards the gram-positive bacteria L. monocytogenes ATCC 35152 compared to the gram-negative bacterium S. boydii ATCC 9905.

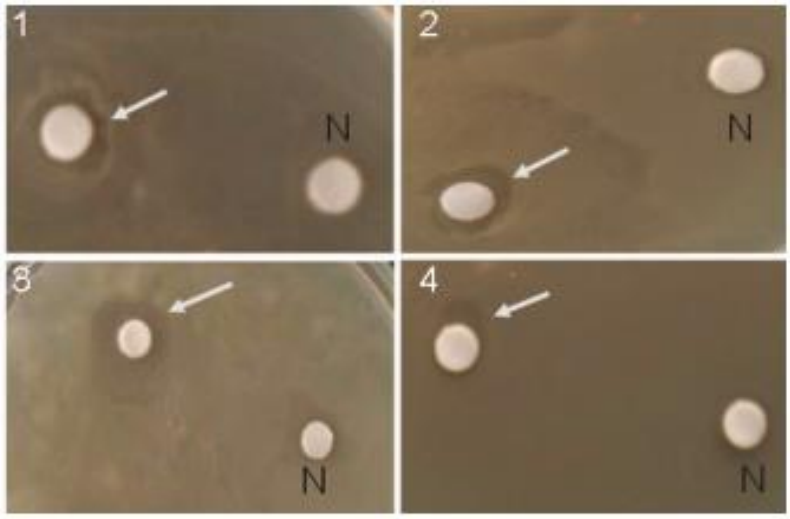

Figure 4: Antibacterial Activity of StL-20 against E. coli

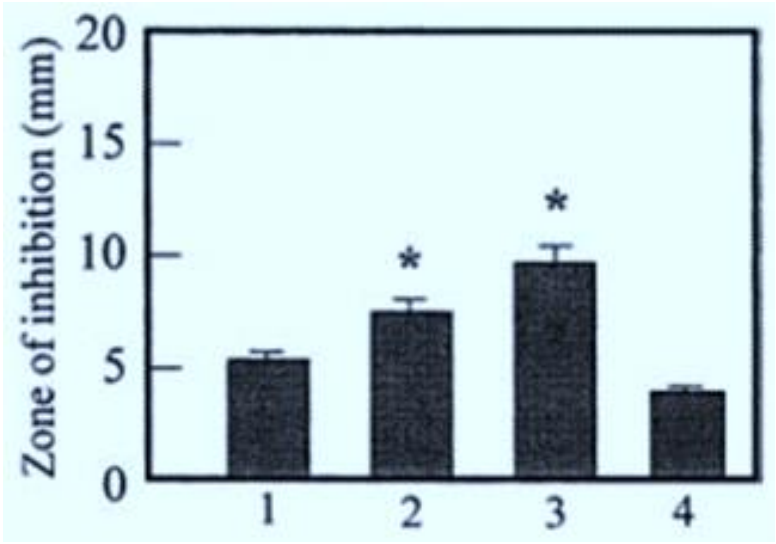

Figure 5: Gram-positive bacteria L. monocytogenes ATCC 35152 compared to the gram-negative bacterium S. boydii ATCC 9905

Antifungal activity

Figure 6 demonstrates the inhibitory effect of StL-20 on the growth of three species of fungal infections: Rhizopus spp., Penicillium spp., and Aspergillus niger. When A. niger was compared to other fungi (Fig. 7, columns 2 and 3), it exhibited a significant vulnerability to StL-20.

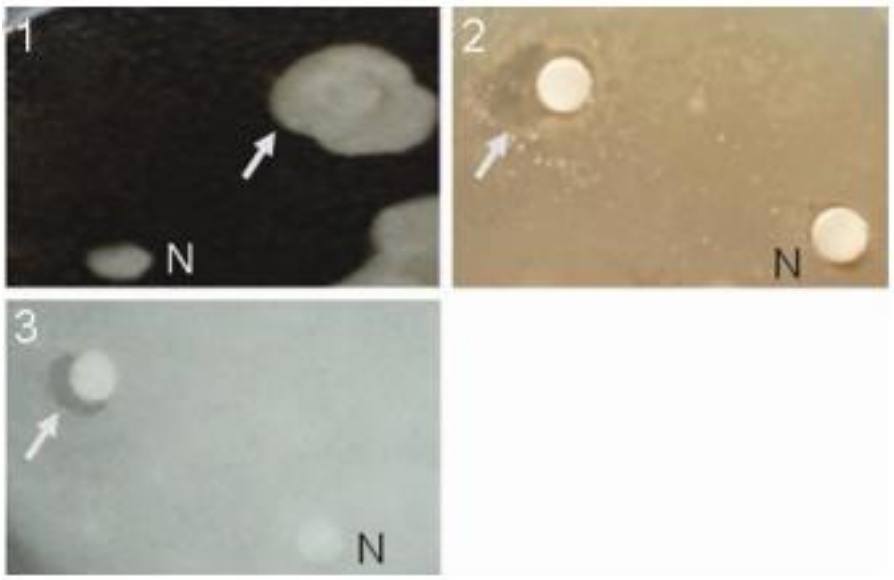

Figure 6: Inhibitory effect of StL-20 on the growth of Rhizopus spp., Penicillium spp. and A. niger

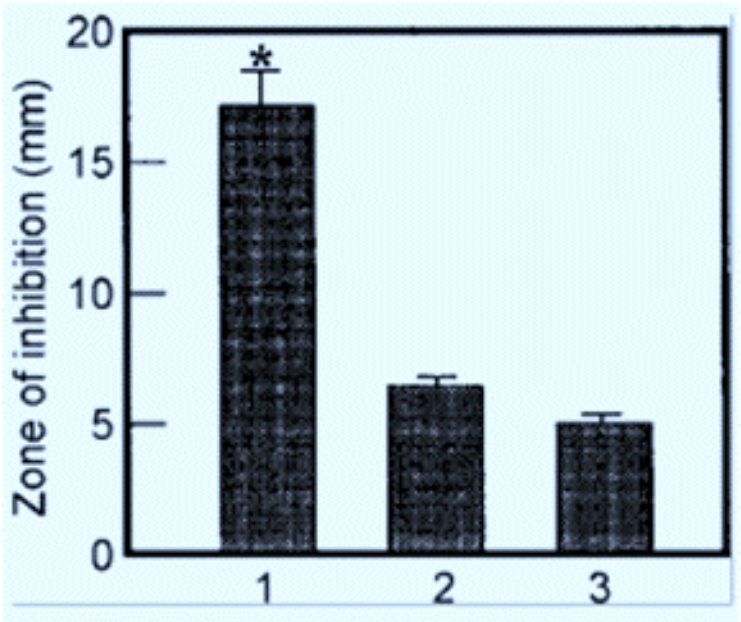

Figure 7: A. niger compared with other fungi

V. CONCLUSION

This work demonstrated the possibility of a bioactive molecule with antibacterial and antifungal effects by successfully isolating and characterizing a new chitin-binding lectin from the 'Deshi' potato cultivar. The 20,000 Daltons lectin effectively killed both Gram-positive and Gram-negative bacteria, including

Listeria monocytogenes and Escherichia coli, Salmonella enteritidis, and Shigella boydii. Also, it demonstrated strong antifungal action against common types of fungi, such as Aspergillus niger, Rhizopus spp., and Penicillium spp.

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