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COMPARISION OF ANTIFUNGAL ACTIVITY BETWEEN PEA AND MUSHROOM LECTINS

Biotechnology

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ABSTRACT

Lectins play vital roles in biological processes. In this paper comparison is done between two different lectins and their role in antifungal activities crude extraction and purification process are explained in this paper. Mushroom and pea lectins obtain health enhancing and pharmaceutical importance worldwide. Most lectins that bind glucose interact equally with N-acetylglucosamine and acetamide group which enhance the binding activity. Many species of lectins specifically bind the sugar N-acetylglucosamine, its chitin, and oligomers were proved to contain antifungal activity. Activity was compared between other lectin carbohydrate-binding property. that might endow lectin molecules with binding activity towards particular carbohydrates. components in fungal cell wall affects its activity and viability as most lectins recognize either N-acetylneuraminic acid, N-acetylglucosamine, N-acetylgalactosamine, galactose, mannose, or fucose . clear zone of inhibition surrounding the sample shows the inhibitory power of the sample against the particular test.

KEYWORDS

Antifungal, Lectins, Pea, Mushroom.

INTRODUCTION

Lectins are small subunits these are naturally occurring glycoproteins and the molecular weight is below 40kDa. *Pisum sativum* (Pea lectin) molecular weight is 49kDa and are dimers and contains carbohydrate-binding site. It contains antiparallel strands and interconnected with loops and turns.

A. *bisporus* also known as edible mushroom has proteins and has therapeutic interest this belongs to ABL and Abmb and has anti-proliferative activities towards cancer cells.it contains three polysaccharides, fatty acids, and phenolic compounds. Besides this, these antioxidants contain a variety of secondary metabolites.Most of the mushroom are dimeric and some are monomeric, trimeric or tetrameric.sugar contents will range from 0 to 18%.These lectins also contain immunomodulatory activities.[2]

MATERIALS AND METHODS

Lectin source

Garden pea was obtained from the agricultural research center, Giza Egypt. [1]

A. *bisporus* procured from DMR, solan (H.P) Shimla.[6]

10 grams of dried seeds were then soaked in the water overnight at 4°C, then homogenised in 100ml of 0.05M (Tris–HCl) buffer, pH 8.0, containing 1mM of CaCl2, 1mM MgCl2 and 1mM MnCl2, and 0.02 percent NaN3 with mixer for about 30 seconds, and then it was stirred for about 30 seconds followed by an ultraturrax for another 30 seconds, and stirred for around 2 hrs at 4°C. The suspension was then filtered through nylon tissue and centrifuged for 20 minutes at 23,500g. by adding 1 M acetic acid to this stirred solution until pH 5.0 was achieved, storage proteins were precipitated. The suspension was recentrifuged (20min at 23,500g) after an additional hour of stirring at 4°C, and the supernatant was readjusted to pH 8.0 with the suitable aliquot of 1M NaOH. The latter solution was dialyzed repeatedly using dialysis bag with the same buffer.[1]

Antifungal activity performed on the sterile Petri plates 100 to 15 mm containing 10ml Dox's agar 1000ml DW, NaNO2 3.0 g, K2HPO4 1.0g, MgSO4 7 H2O 0.5g, FeSO4 7 H2O, 0.01g, Sucrose 30.0g, Agar 15.0g. sterilized at 15 psi and 120 °C for 20 min in an autoclave. Sterile paper disks 1cm in diameter, were used at the surface of heavily seeded medium with the tested organism. A 0.01ml aliquot of lectin which is purified in 0.05M Tris–HCl buffer pH 8.0 containing 0.15 M NaCl was added to the disk. The diameter of the clear zone of inhibition that formed around the sample was taken as a measure of the inhibitory power of the sample against the particular test organism after 5 days of incubation at 25–27°C.[1]

Mushroom crude extraction and purification

Fruiting bodies of mushroom were dried in shade conditions and dried material 50g was small-grained in a blender to get fine powder and soaked separately in 300ml of methanol and acetone Erlenmeyer flask used for methanol and acetone extracts. The flasks were covered with aluminum foil and then allowed to stand for 7 days for crude extraction. These extracts were filtered through Whatmann filter paper and evaporated at 40°C using a rotary evaporator. The extracts were collected and then a stock solution of conc.10 mg/ml was prepared. Purification was done by ammonium sulfate precipitation and dialyzed.

The fungal strains of Candida and Fusarium species were cultured at 28°C in yeast extract peptone dextrose and potato dextrose broth medium. fungal cells (final concentration, 2×104 colony-forming units/mL) in 50 µL of corresponding media were seeded in each of the wells of a microtiter plate containing 50 µL of serially diluted (2-fold lectin in a buffer. After incubation for 24–30 hours at 28°C, MIC values were microscopically determined. All MIC values were determined by averaging the values of 3 or 4 independent sets of experiments. All assays were performed triplicate-wise. To visualize the fungicidal effect, morphological changes in the sample were examined by phase-contrast light microscopy by using an ECLIPSE TE300 microscope.[6]

RESULT

1. Mushroom lectin

The zone of inhibition was calculated by measuring the diameter of the zone around the well (in mm) along with the well diameter. The readings were taken in the perpendicular direction of all three replicates and the average was taken. The percentage of inhibition of growth of bacterial microorganisms was calculated by subtracting control from the values of inhibition diameter taking control as standard.

Percentage of growth inhibition= (Control- Test/Control) x100
Control=average diameter of a bacterial colony in control.
Test=average diameter of a bacterial colony in treatment sets.

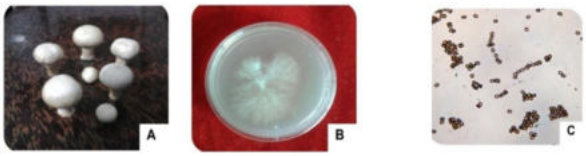

Fig.1 Fruiting bodies of Agaricus bisporus (A)Pure culture of Agaricus bisporus (B) Basidiospores of Agaricus bisporus (C) Basidiospores of Agaricus bisporus

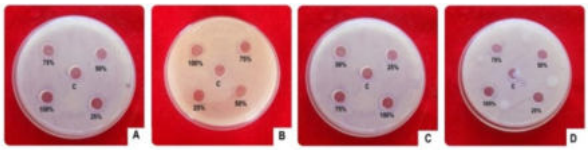

Fig.2(A) Inhibition in the growth of S.aureus at different concentrations of methanolic extract, (B) Acetone extract of Agaricus bisporus. (C) Inhibition in the growth of E. coli at different concentrations of methanolic extract, (D) Acetone extracts of Agaricus bisporus.

2. Pea lectin

In the end, the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test. The observed antifungal activity of PSL against A. flavus, T. viride, and F. oxysporum co ordinates with the other results obtained from other plant legume lectins.[1] Activity was concluded to be related to the lectin carbohydrate-binding property. that might endow lectin molecules with binding activity towards particular carbohydrates. components in fungal cell wall affects its activity and viability as most lectins recognize either N-acetylneurami- nic acid, N-acetylglucosamine, N-acetylgalactosamine, galactose, mannose, or fucose .

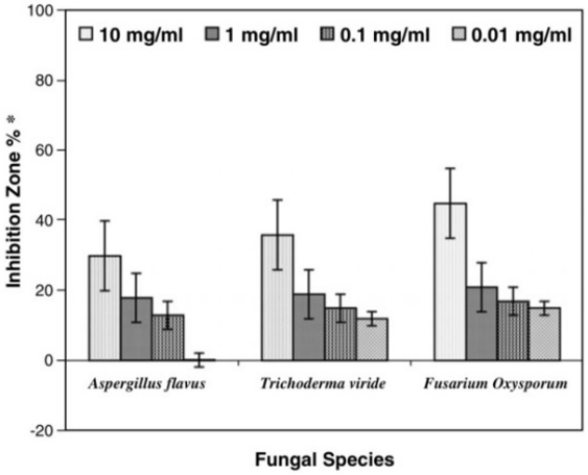

Fig.3 Antifungal activity of *Pisum sativum* lectin showing inhibition zone divided by the total plate zone (90mm).

CONCLUSION

Most lectins that bind glucose interact equally with N-acetylglucosamine and acetamide group enhance the binding activity. many species of lectins, which specifically bind the sugar N-acetylglucosamine, its oligomers, and chitin were proved to contain antifungal activity. Many pharmaceutical substances with potent health-enhancing properties have been isolated from mushrooms and pea worldwide.

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