Biolife 2(4):1354-1358
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

Evaluation of antifungal potential of chitin and chitin-based derivatives against pathogenic fungal strains

Junaid Alam[1]* and Abhishek Mathur[2]

[1]*Himalayan University, Arunachal Pradesh, India.*
[2]*Institute of Transgene Life Sciences, Dehradun (U.K), India.*

E-mail: junaidbiotechnology@hotmail.com

ABSTRACT

Chitin, a predominant polysaccharide besides cellulose is present as an important constituent of exoskeleton of arthropods and crustaceans. It is also found as the important component of fungal hyphae. Chitin and its derivatives had a great significance and importance in pharmacological, medical and food dealing fields. In the present investigation, chitinous wastes were collected from the fresh water areas of Dehradun and Rishikesh of Uttarakhand State. The bacterium, Bacillus sp. isolated from soil produces chitinase enzyme responsible for degradation of chitin obtained from chitinous wastes. Further the chitinases enzyme was utilized to degrade the chitinous wastes into chito-oligosaccharides. The chitin active molecule present in the chitinous waste at another stage was deacetylated to chitosan. Further the antifungal activity of chitinases, chitin, chitosan and chito-oligosaccharides was determined in vitro by well diffusion method against Aspergillus niger and Candida albicans. Amongst the test fungal cultures, the chitinase showed maximum inhibition against Aspergillus niger (diameter of zone of inhibition: 24 mm) followed by Candida albicans (diameter of zone of inhibition: 14 mm). Deacetylated form of chitin i.e chitosan showed potent antifungal activity against Candida albicans (diameter of zone of inhibition: 24 mm) followed by Aspergillus niger (diameter of zone of inhibition: 18 mm). The chitin extracted showed almost similar antifungal activity against Aspergillus niger and Candida albicans (diameter of zone of inhibition: 15 mm) respectively. The low molecular weight derivatives viz. chito-oligosaccharide showed significant antifungal activity against Aspergillus niger (diameter of zone of inhibition: 14 mm) but no activity was found against Candida albicans.

Keywords: Chitin, crustaceans, chito-oligosaccharides, chitosan, chitinases, antifungal activity

INTRODUCTION

Chitin, a polysaccharide of animal origin, is obtained from waste material of seafood industries. It occurs in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. Chitin is also the important component of exoskeleton of Arthropods Chitin is also forming the important composition of fungus. Chitin hold great economic value due to their versatile biological activities and chemical applications, mainly in medical (Murugan et al., 2004; Yadav and Bhise, 2004) and pharmaceutical areas (Takeuchi et al., 2001; Kato et al., 2003). Chito-oligosaccharides and their N-acetylated analogues are useful for applications in various fields because they have specific biological activities such as antimicrobial activity, antitumor activity, immune-enhancing effects (Gohel et al., 2006).

Some chito-oligosaccharides such as (GlcNAc) and (GlcNAc) have been reported to possess antitumor activity (Suzuki et al., 1986; Liang et al., 2007). Chitinolytic enzymes have been widely used in various processes including the agricultural, biological and environmental fields (Chuan, 2006). Several chitinolytic enzymes have been identified in various Streptomyces sp., including, Streptomyces plicatus (Robbins et al., 1988), S. lividans (Miyashita et al., 1991), S. virdificans (Gupta et al., 1995) and S. halstedii (Joo, 2005). The chitinases were purified and characterized from marine bacterium (Lee et al., 2000). The potent chitinolytic activity of marine actinomycetes species and enzymatic production of chito-oligosaccharides was investigated (El-Shayeb et al., 2010). In the present study, the antibacterial activity of chitin and chitin based derivatives was determined in lieu of determination of pharmacological properties present in the abundant polymer.

MATERIALS AND METHODS

All the materials, reagents and media used for the study were procured from Ranchem, CDH and Hi-Media, India.

Collection of chitinous wastes:
The chitinous wastes of fresh water crustaceans were collected from the fresh water areas of Dehradun and Rishikesh (U.K), India and were washed properly in order to remove the sand debris present on the surfaces. The chitinous wastes were then after air dried and powdered material obtained was used as chitin.

Demineralization of chitinous wastes:
The demineralization of chitinous wastes was performed (Gagne and Simpson, 1993; Mathur et al., 2011). The chitinous wastes were treated with 1.75 N acetic acid at room temperature for about 12-15 hours. The ratio of waste to solvent were maintained (1:15 w/v). The demineralized material obtained were recovered by filteration and rinsing with de-ionized water and will be dried in forced hot air oven at 650C.

Deproteinization and removal of lipids:
The new and advanced methodology for deproteinization of proteins from demineralized chitinous wastes was designed by using deproteinization agents. This process can be performed either by using proteolytic enzymes such as proteinase-K dissolved in buffer containing 0.05 M Tris-base (pH, 6.5-9.1) in a ratio 1:20 (w/v) in flasks at various temperatures in incubator-shaker for about 72 h and adding mixture of solvents (phenol: chloroform, 1:1 ratio) again and again to the residue obtained and centrifuging the mixture until the residue gives no test for the presence of protein content. After repeating the procedure for 3-4 times, finally the residue was treated with 2N sodium hydroxide (1:25 w/v) at 700C for 1 hour. The lipid content gets dissolved in phenol: chloroform mixture and was removed from the chitinous wastes. Greese spot test can be performed in order to determine qualitatively the presence of lipid content if any present in the residual material (Mathur et al., 2011).

Preparation of colloidal chitin:
The colloidal chitin was prepared by using 1g of standard chitin, fresh water crustaceans chitin separately in 1N HCl for 2 h at room temperature. The colloidal chitin prepared of each of the samples was washed several times with large volume of distilled water to adjust the pH to 7.0.

Isolation of microorganisms for screening of chitinases production:
The soil was procured from Doiwala region of Dehradun (U.K), India by performing the serial dilution method and maintained on glycerol yeast medium plates at 37°C.

Screening and culture conditions:
For the screening purpose, strain was inoculated in 100 ml of medium (3% w/v chitin, 0.1% KH2PO4, 0.05% MgSO4.7H2O, 50 mM Sodium Phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C. Further basic dye cresol red was added in a flask and kept for 18-24 h. The conversion of colour of the red dye into purple (pH, 6.5- 8.8) was taken as an indication for the presence of Bacillus sp. Further biochemical tests such as amylase

production/starch hydrolysis assay were performed in order to confirm the strain as Bacillus sp. Gram staining confirmed the strain as gram positive (Mathur et al., 2011).

Chitinase Production :

For the production of chitinase, strain was grown in 100 ml of fresh medium (3% w/v chitin; 0.1% KH2PO4; 0.05% MgSO4.7H2O; 50mM Sodium Phosphate buffer, pH 6.0) in a 250 ml Erlenmeyer flask at 30°C. For reflecting the growth of the culture in this medium OD at 660 nm was taken using blank as medium in which no inoculum was added. The supernatant (enzyme) was collected from 3 day old cultures by centrifuging the mixture at 12,000 g for 20 minutes. The enzyme was concentrated by condensing the solution in order to reduce its volume (Mathur et al., 2011).

Preparation of Chito-oligosaccharides:

For the preparation of chito-oligosaccharides, 1% colloidal chitin prepared from standard chitin, fresh water crab and fresh water prawn were dissolved in 0.05M Phosphate buffer (pH, 5.5). About 10 ml of enzyme was added in 15 ml of 1% of each of the colloidal chitin in a 100 ml flask; further flask was kept at 30°C for 3 h. The reaction was terminated by immersing the tubes in boiling water for 5 minutes. After performing centrifugation at 3000 rpm, the insoluble materials were collected and condensed to obtain the chito-oligosaccharides (Mathur et al., 2011).

Preparation of Chitosan:

The demineralized and deproteinized chitin material was subjected to concentrated sodium hydroxide at 40% w/v (Alam et al., 2013; Imoto and Yagishita, 1971). The deacetylated forms of chitosan obtained were solubilized in 2 M dilute acetic acid.

Determination of antimicrobial activity of chitin and chitin-based derivatives produced from strain:

The antimicrobial activity of chitinase produced from Bacillus strain and chitin-based derivatives were screened for its antibacterial activity against some standard fungal strains viz. Aspergillus niger and Candida albicans by well diffusion method (Perez and Anesini, 1993). The pure cultures of test microorganisms were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India. Sabouraud's dextrose agar/broth was used for the growth of fungal cultures. The wells were punctured in the agar plates with sterile borer and 105 Cfu/ml of the fungal cell suspension were introduced in the plates separately. The enzyme supernatant was introduced in the wells in each of the bacterial and fungal plates. The plates were left free for the thorough diffusion of the enzyme supernatant within the medium plates and were kept for 72 h at 370 C for fungal cultures. The diameter of zone of inhibition observed was recorded.

RESULTS AND DISCUSSION

In the present investigation, the antifungal activity of chitinase enzyme and chitin-based derivatives was determined. The results were found to be very significant. The chitinase showed maximum inhibition against Aspergillus niger (diameter of zone of inhibition: 24 mm) followed by Candida albicans (diameter of zone of inhibition: 14 mm). Deacetylated form of chitin i.e chitosan showed potent antifungal activity against Candida albicans (diameter of zone of inhibition: 24 mm) followed by Aspergillus niger (diameter of zone of inhibition: 18 mm). The chitin extracted showed almost similar antifungal activity against Aspergillus

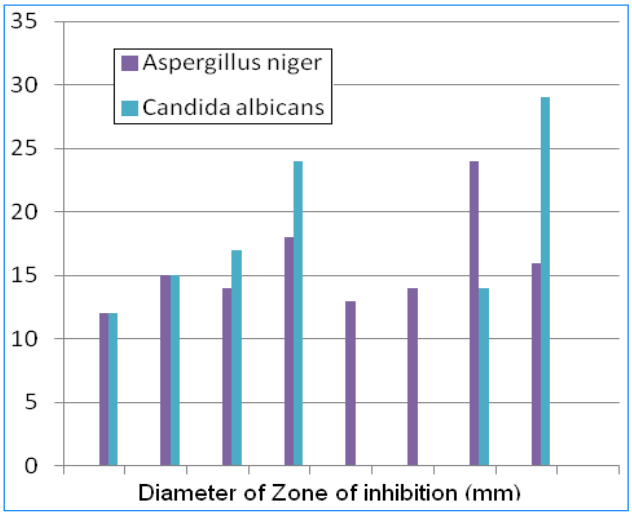
Table 1: Anti-fungal activities of Chitinase, Chitin and Chitin Based Derivatives

Fungal Strains	Diameter of Zone of Inhibition (mm)								
	Standard Chitin	Extracted Chitin	Standard Chitosan	Extracted Chitosan	Standard Chito-oligosaccharide	Extracted Chito-Oligosaccharide	Chitinase	Flucanazole	Negative Control 0.1% NaOH
Aspergillus niger	12	15	14	18	13	14	24	16	NA
Candida albicans	12	15	17	24	NA	NA	14	29	NA

*NA, No activity

niger and Candida albicans (diameter of zone of inhibition: 15 mm) respectively. The low molecular weight derivatives viz. chito-oligosaccharide showed significant antifungal activity against Aspergillus niger (diameter of zone of inhibition: 14 mm) but no activity was found against Candida albicans. The results are shown in Table 1 and Figure 1(a) & (b).

Figure-1 (a): Anti-fungal activities of Chitinase, Chitin and Chitin Based Derivatives.



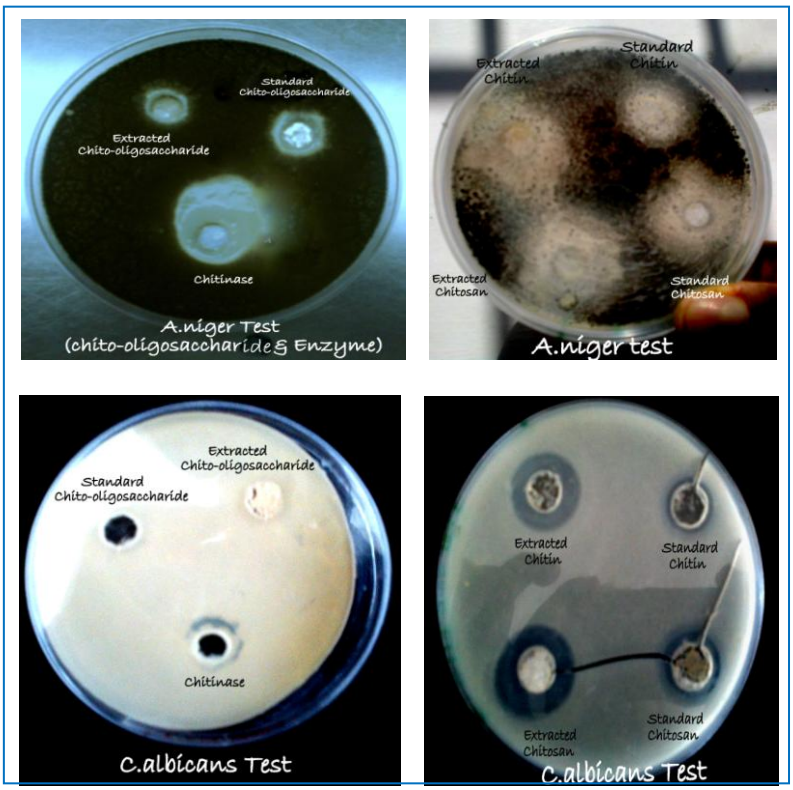
CONCLUSION

The results hereby suggest that, chitinase enzyme and chitin-based derivatives are potent antifungal agent against Aspergillus niger and Candida albicans. The study thus emphasizes the use of these molecules as antifungal agents and these substances can be utilized in anti-fungal medicines and drugs. Further researches are required to isolate the gene of interest of chitinase which can be expressed in plants to combat the infection caused by pathogenic fungi.

REFERENCES

1. Alam J, Kushwaha A, Mathur A. 2013. Evaluation of growth regulatory effect of chitinand chitin-based derivatives extracted from fresh water crustaceans.Recent Res. Sci. Tech. 5(1), 05-08.
2. Chuan LD. 2006. Review of fungal chitinases. Mycopathologia. 161, 345-360.
3. El-Shayeb NA, Hosny MS, El-Dein A. 2010. A potent chitinolytic activity of marine actinomycetes species and enzymatic production of chito-oligosaccharides.

Figure-1 (b): Anti-fungal activities of Chitinase, Chitin and Chitin Based Derivatives against *A. niger* and *C. albicans.*



Australian J. Basic and Applied Sciences. 4(4), 615-623.
4. Gagné N and Simpson BK. 1993. Use of proteolytic enzymes to facilitate the recovery of chitin from shrimp wastes. Food Biotechnol. 7, 253-263.
5. Gohel V, Singh A, Maisuria V, Phdnis A and Chatpar HS. 2006. Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr. J. Biotechnol. 5, 54-72.
6. Gupta R, Saxena, RK, Chaturvedi P and Virdi JS. 1995. Chitinases production by Streptomyces virdificans, it's potential in fungal cell walls lysis. J. Appl. Bacteriol. 78, 378-383.
7. Imoto T and Yagishita K. 1971. A simple activity measurement by lysozyme. Agric Biol. Chem. 35, 1154– 1156.
8. Joo GJ. 2005. Purification and characterization of an extracellular chitinase from the antifungal biocontrol agent Streptomyces halstedii. Biotechnol Letters. 27, 1483- 1486.
9. Kato Y, Onishi H and Machida Y. 2003. Application of chitin and chitosan derivatives in the pharmaceutical field. Curr. Pharm. Biotechnology. 4(5), 303-309.
10. Lee HK, Lee JH and Park SH. 2000. Purification and characterization of chitinases from marine bacterium Vibrio sp.98CJ11027. The J. Microbiology. 38(4), 224-229.
11. Liang TW, Chen YJ, Yen YH and Wang SL. 2007. The antitumor activity of the hydrolysate of chitinous materials hydrolysed by crude enzyme from Bacillus amyloliquefaciens V656. Process Biochem. 2, 527-534.
12. Mathur A, Rawat A, Bhatt G, Baweja S, Ahmad F, Grover A, Madhav K, Dhand M, Mathur D, Verma SK, Singh SK, Dua VK. 2011. Isolation of Bacillus producing chitinases from soil: Production and purification of chito-oligosaccharides from chitin extracted from fresh water crustaceans and antimicrobial activity of chitinases. Recent Res. Sci. Tech. 3(11), 01-06.
13. Miyashita K, Fujii T and Sawada Y. 1991. Molecular cloning and expression of a Streptomyces lividans. J Gen Microbiol. 137, 2065- 2072.66.
14. Murugan R. and Ramakrishna S. 2004. Bioresorbable composite bone paste using polysaccharide based nanohydroxyapatite. Biomaterials. 25(17), 3829-3835.
15. Perez C and Anesini C. 1993. In vitro antimicrobial activity of Argentine folk medicinal plants against Salmonella typhii. Journal of Ethnopharmacology. 44, 41-46.
16. Robbins PW, Albright C and Benfield B. 1988. Cloning and expression of a Streptomyces plicatus chitinases (chitinase-63) in Escherichia coli. J Biol. Chem. 263, 443- 447.
17. Suzuki K, Mikami T, Okawa Y, Tokoro A, Suzuki S and Suzuki M. 1986. Antitumor effect of hexa- Nacetylchitohexaose and chitohexose. Carbohydr. Res.151, 403-623.
18. Takeuchi H, Yamamoto H and Kawashima Y. 2001. Mucoadhesive nanoparticulate systems for peptide drug delivery. Adv. Drug Deliv. Rev. 47(1), 39-54.
19. Yadav AV and Bhise BB. 2004. Chitosan a potential biomaterial effective against typhoid. Curr. Sci. 187(9), 1176-1178.

*****