

## Study of enzyme chitinase produced by *Bacillus subtilis* and its antifungal activity against *Aspergillus species*



### Microbiology

**KEYWORDS :** *Bacillus subtilis*; *Aspergillus species*; Chitin; Chitinase; Spectrophotometer

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### ABSTRACT

*In the present investigation, the culture of Bacillus subtilis was obtained from NCL, Pune. Chitin is used as a substrate for the production of enzyme Chitinase. Colloidal chitin was prepared by using chitin flakes as well as concentrated Hydrochloric acid and cold ethanol. This colloidal chitin was used for the screening of chitin degradation. The activity of chitinase production was optimized with the study of culture condition like effect of medium, pH, temperature and incubation time on chitinase production. The maximum chitinase activity was showed in Nutrient Agar Medium at pH 8.0 and at temperature 40° C after three days of incubation period. Chitinase assay was performed to nullify the chitinase producing ability of microorganisms. The antifungal activity was assayed in vitro by inhibiting the growth of fungus on Sabouraud's Dextrose Agar medium. After three days of incubation the Antifungal activity was shown against Aspergillus flavus, Aspergillus niger.*

### Introduction

Chitin, a second most abundant organic compound and it is a polysaccharide of animal origin. It is also occurs in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. Interestingly the presence of chitin in the cyst wall of human pathogen<sup>[1]</sup>. Chitinase is the chitinolytic enzyme which is responsible for the complete degradation of chitin. Chitinase are produced by several bacteria<sup>[2]</sup> actinomycetes<sup>[3]</sup>, fungi and also by higher plants.<sup>[4]</sup> The enzyme chitinase are used in various applications such as biological control of fungal pathogen<sup>[5]</sup> <sup>[6]</sup>, preparation of pharmaceutically important chito-oligosacchride and N-acetyl-D-Glucosamine<sup>[7]</sup>. Chitinase may be used to treat fungal infection in human being such as cutaneous mycoses, subcutaneous mycoses, pulmonary mycoses and candidiasis<sup>[8]</sup>. Microorganism produces the chitinase primarily for assimilation of chitin as carbon or nitrogen source<sup>[9]</sup>. In this present study chitinase activity was shown by *Bacillus subtilis* as well as antifungal activity against *Aspergillus species*.

### Material and Method

Preparation of colloidal chitin: Colloidal chitin was prepared by using chitin flakes. 1gm of chitin flakes was added slowly into 18 ml of concentrated HCL under vigorous stirring; 100 ml of ice cold ethanol was added with rapid stirring and kept it overnight at 25° C with the use of centrifuge machine. The precipitate was collected by centrifugation at 6000 rpm for 10 min at 4° C. Then it was washed with sodium phosphate buffer and maintained at pH 7.

### Screening for Chitin Degradation:

The culture of *Bacillus subtilis* was grown in Nutrient Broth containing 0.1% colloidal chitin and it was incubated at 100 rpm in a rotary shaker at room temperature for 2 days. After that, culture was centrifuged at 1000 rpm 15 min at 4° C and supernatant was collected. CCA (Colloidal Chitin Agar) Plates were prepared & dipped a plain disc in the culture filtrate and placed it in the agar and incubated at 37° C. After 12 hrs the development of clear zone around the disc was observed.

### Optimization of culture condition: -

#### Effect of Medium on chitinase production:

Two different medium such as Nutrient Broth & Luria Bertaini Broth is used. In which 0.1% colloidal chitin was added and stirred. The culture was inoculated and incubated at 37° C for 24 hrs and the chitinase production was observed by using spectrophotometer at 540 nm.

#### Effect of pH on chitinase production :

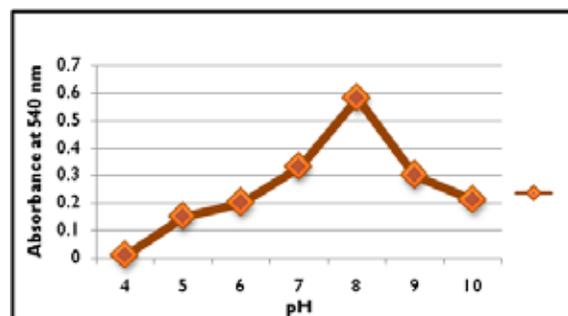
The *Bacillus subtilis* inoculated in LB Broth at different pH

ranges from pH 4 to 10 and incubated at 37° C for 24 hours. The chitinase production was observed in spectrophotometer at 540 nm.

**Table No:- 1 Effect of pH on chitinase production**

Sr.No.	Buffer at different pH	O.D. at 540 nm
1	4	0.01
2	5	0.15
3	6	0.02
4	7	0.33
5	8	0.58
6	9	0.30
7	10	0.21

**Graph :- 1 Effect of pH on chitinase production**



#### Effect of Incubation Time for chitinase production:

The *Bacillus subtilis* inoculated in LB Broth at different incubation period from 1 to 7 days and incubated at 37° C for 24 hrs. The chitinase production was observed in spectrophotometer at 540 nm.

**Table No:- 2 Effect of Incubation Time for chitinase production**

Sr. No.	Incubation Day	O.D. at 540 nm
1	1	0.56
2	2	0.73
3	3	1.10

4	4	1.06
5	5	1.04
6	6	0.95
7	7	0.92

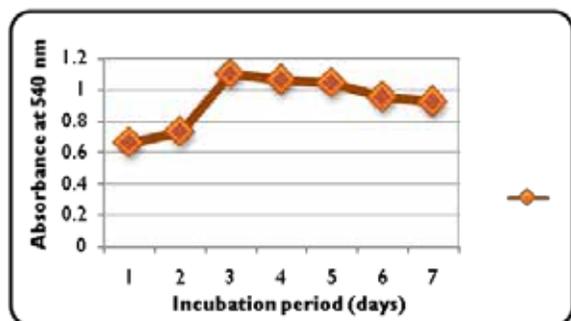


Figure : 2- Effect of Incubation Time for chitinase production

**Assay of chitinase production:** A chitinase assay was performed to nullify the chitinase producing ability of microorganisms. The crude enzyme from culture supernatant was incubated with 2ml of chitin substrate was kept for 2 hours at 25°C with vigorous shaking. After incubation period, the reaction mixture was boiled for 5 minutes. After boiling 2ml substrate was added to the control without incubation, followed by centrifugation at 4500 rpm for 10 minutes. 1ml of supernatant, 1.5 ml of DNSA was added and boiled for 5 minutes in a water bath, cool at room temperature and colour produced was read at 540 nm in a spectrophotometer.

**Antifungal activity:** The antifungal activity was assayed in vitro by inhibiting the growth of fungus on Sabouraud's Dextrose Agar medium. SDA medium were prepared and sterilized and poured into the petri plates. After solidification, the fungal colonies were inoculated on the SDA medium, after 30 minutes a paper disc of *B.subtilis* placed on petri plate and incubated at room temperature. After three days of incubation, the diameter of inhibition was measured and expressed in mm.

**Result and Discussion:** Microorganisms which secrete a complex of mycolytic enzymes are considered to be possible biological control agents of plant diseases. Since chitinases are digestive enzymes that breakdown glycosidic bonds in chitin. In this present study, *Bacillus subtilis* was found to be efficient for the production of chitinase. An extracellular chitinase was secreted by *Bacillus subtilis* in a medium when supplemented with colloidal chitin [1%]. The enzyme chitinase was produced by the degradation of chitin efficiently at optimum pH and temperature. The culture medium is key factor for the growth as well as metabolites production by microorganisms. In this present study it was observed that Nutrient Broth give high chitinase production than Luria Bertaini Broth as well as the efficient chitinase production was observed at optimum pH 8 and temperature 40°C after 3 days of incubation. The concentration of colloidal chitin is another vital factor as it is reported to induce the chitinase production in several microorganisms<sup>[10]</sup>. The chitinase activity was showing the ability of chitinase production and also shows antifungal activity.

Figure:- 1 Chitinase activity shown by *Bacillus subtilis*



Figure:- 2 Antifungal activity shown by *Bacillus subtilis* against *Aspergillus flavus*



**Conclusion:** From the present investigation it was confirmed that the *Bacillus species*, such as *Bacillus subtilis* have chitinase activity and thus the enzyme extracted from this species can be used as a catalyst for the degradation of chitin which is an abundant polysaccharide after starch. Chitinase enzyme has been widely used in various processes including agricultural biological and environmental fields. From our studies it is revealed that chitinase having potent antifungal activity, thus there is need have to commercialize the production of chitinase enzyme from such microbial organisms which have beneficial aspects in pharmaceutical and food industries.

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