

## Research article

# Extraction and purification of chitinase from *Bacillus subtilis*

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### ABSTRACT

Four isolates of *Bacillus subtilis* produced chitinase were isolated from soil. The bacterial isolates were grown in liquid medium supported with different salts and nitrogen sources. The highest produced isolate was selected for further experiments. Different method was used to purify the enzyme. The optimum condition for enzyme activity was evaluated in present study. *B. subtilis* A3 gave the highest enzyme production. The different purification steps were followed to purified casein from *B. subtilis* A3. The produced enzyme was precipitated at 80% of saturated solution of ammonium sulphate. The salt was eradicated by dialyzed the yielded enzyme with distilled water. Finally, the enzyme solution was run through ion-exchange chromatography (Sephadex G-100 column). The maximum specific activity (5.1 U/mg of protein) was observed in liquid medium supported with casein and incubated in 30 °C with pH 8.

**Keywords:** *Bacillus subtilis*, chitinase, Ion-exchange chromatography, pH, temperature.

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## INTRODUCTION

Chitinase is the most important enzyme in the field of bio-technology for its importance in the control of diseases caused by insects and fungi to humans, animals and plants [1]. These enzymes divided depending on the kind of the break of bounds, endochitinase that breaks the internal bounds, while that breaks peripheral bounds called the exochitinas [2]. Several studies reported the ability of *Bacillus* to produce chitinase that breaks the chitins to produce

A-acetyl glucose amine in cultured media [3]. The other studies reported many genus could produce the chitinase [4]. Chitinase plays a certain role in the host that dependent on the chitin as the main source of carbon [5]. The production and activity of the enzyme are dependent on many factors such as temperature, pH and components of growth media. Chitinase used in different industrial fields, such as pharmaceutical and food industries. This



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enzyme s highly important in environment, because the ability of this enzyme to analyze the skeletons of insects [6]. In current study, we try to produce chitinase from *B. subtilis* and find the best condition for producing and activity of this enzyme.

## **MATERIALS AND METHODS**

### **Bacterial strains**

Four isolates of *B. subtilis* were isolated from soil. The standard method was followed to identification of bacteria. The isolates were stored in nutrient agar slant for one month and routinely sub-cultured in nutrient agar plates for maintaining the isolates.

### **Culture media**

Liquid media: The special liquid medium was used to maintain the bacteria and produce the enzyme. This media composed of NaCl (0.5 g), MgSO<sub>4</sub> (1 g), Yeast extract (0.5 g), Colloidal chitin (0.5 %), K<sub>2</sub>HPO<sub>4</sub> (1 g) and Trypton (0.5 g) dissolved in 100 ml of distilled water, the pH of solution was adjusted to 8 and sterilized by autoclave (121°C, 1 bar or 15 lb/sq. in., for 15 min). Similar formula was followed to prepare other media except tryptone was changed each time with different nitrogen sources (casein, peptone, gelatin, tryptone and yeast extract) to check the best media for enzyme production.

### **Solid media**

YEPD medium was used to check the ability of isolates to produce chitinase. This medium composed of 20 g of glucose, 10 g of yeast extract and 20 g of tryptone, the mixture was dissolved in 100 ml of distilled water and pH was adjusted to 5. The prepared medium was sterilized by heating in an autoclave at 121°C (pressure 1 bar or 15 lb/sq. in.) for 15 min.

### **The factors that affect on enzyme production**

#### **Effect of pH**

The bacteria isolate was re-cultured in liquid medium at 30 °C for overnight. The liquid media were prepared at different pH (4-10). The media were inoculated with 1 ml of overnight cultured media that inoculated previously with isolate that produce the highest level of enzyme. The media was incubated at 30 °C for 6 days. The media were centrifuged and specific enzyme activity was checked in supernatants.

#### **Effect of temperature**

The bacteria isolate was re-cultured in liquid medium at 30 °C for overnight. One ml of growth media was transferred to different flasks contained 100 ml of

liquid media. Inoculated flasks were incubated at different temperatures (20, 25, 30, 35, 40, 45 oC) for 6 days. The specific enzyme activity was checked in supernatants.

### **Extraction of enzyme**

The supernatants were obtained after centrifuging the bacterial growth at 4500 g for 15 minutes. The standard method was followed to check the concentrations of protein and specific activity of enzyme [7, 8].

### **Purification of enzyme**

Ammonium sulphate powder was added to bacterial supernatant (crude enzyme) to obtain 20 % of ammonium sulphate saturated solution. The mixture was mixed and kept in refrigerator for over night. The mixture was centrifuged at 6000 g for 15 minutes. Ammonium sulphate powder was added to supernatant up to 40 % of ammonium sulphate saturated solution. The same procedure was followed up to 80 % of ammonium sulphate saturated solution. The pellet was collected and dissolved in phosphate buffer (0.01 M, pH 8). The solution was dialyzed by dialysis tube against distilled water for 24 h at 4 °C.

### **Ion-exchange chromatography**

The purified enzyme suspension by ammonium sulphate was run through Sephadex G-100 column that washed previously with phosphate buffer (0.01 M, pH 8). The elution rate was adjusted to be 30 ml/h. The protein was ditched from Sephadex G-100 by different concentrations of NaCl (0.2, 0.3, 0.4 and 0.5 M) that dissolved in phosphate buffer (0.01 M, pH 8). The optical density was checked for collected elution buffer at 280 nm. The enzyme activity was checked for elution buffer that gave significant level of OD at 280 nm.

### **Statistical analysis**

All values were calculated as the means ± SD. Differences among multiple groups were analyzed by applying the Tukey honestly significant differences test to a one-way ANOVA using Origin version 8.0 software. A value of  $p < 0.05$  was considered statistically significant.

## **RESULTS**

### **The best bacterial isolate that produce enzyme**

The level of enzyme production was calculated according to specific activity of enzyme that produced in liquid medium supported with casein. All isolates showed good ability to produce the

enzyme but the maximum production was found in isolate *B. subtilis* A3 followed by isolate *B. subtilis* A1, while the minimum enzyme production was observed in case of isolate *B. subtilis* A4 (Fig. 1).

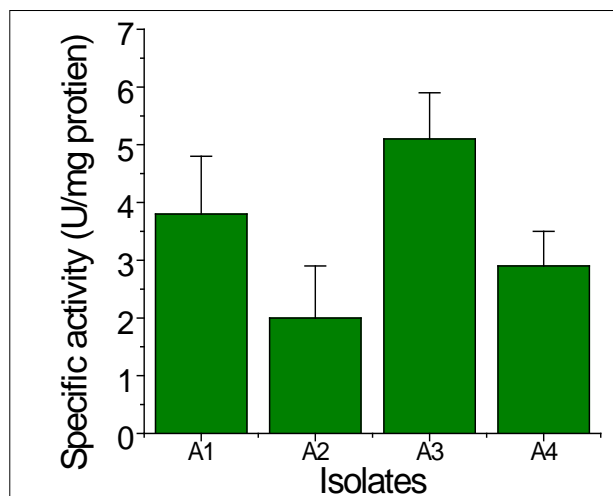


Fig 1. Specific activity of chitinase that produced in liquid product media by different isolates of *B. subtilis*.

#### Effect of different media on enzyme production

The liquid medium that supported with different nitrogen sources was used to check the best enzyme production by *B. subtilis* (A3). Fig. 2 shows the maximum enzyme production was found when the bacterial isolate grown in liquid medium that supported with casein followed by that supported with gelatin, while the lowest enzyme production

when the isolate grown in liquid media supported with tryptone.

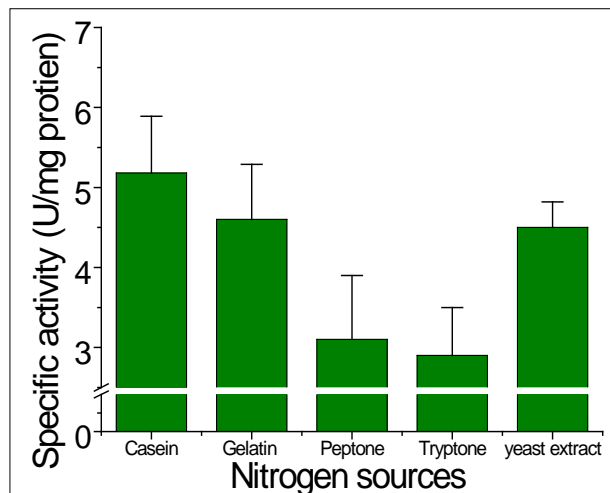


Fig. 2. Specific enzyme activity of *B. subtilis* (A3) grown in liquid media supported with different nitrogen sources.

#### Enzyme production

Table 1 shows the activity of enzyme post different purification methods. The maximum specific enzyme activity was observed post ion-exchange chromatography. The finding was inverted to an enzyme activity. The present study showed that the percentage of enzyme yield was decrease when the purification methods were used. The lowest Percentage of enzyme yield was observed when enzyme purified by ion-exchange chromatography.

Table 1. Enzyme activity, protein level and percentage of enzyme yield for the enzyme at different stages of purification.

Purification steps	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific enzyme activity (U/mg/ml)	Percentage of enzyme yield
Crude enzyme	6.6	1.74	3.8	100
Purified enzyme (ammonium sulphate)	6.9	1.6	4.3	62.6
Purified enzyme (ion-exchange chromatography)	3.3	0.4	8.2	23.3

#### Effect of temperature on enzyme activity

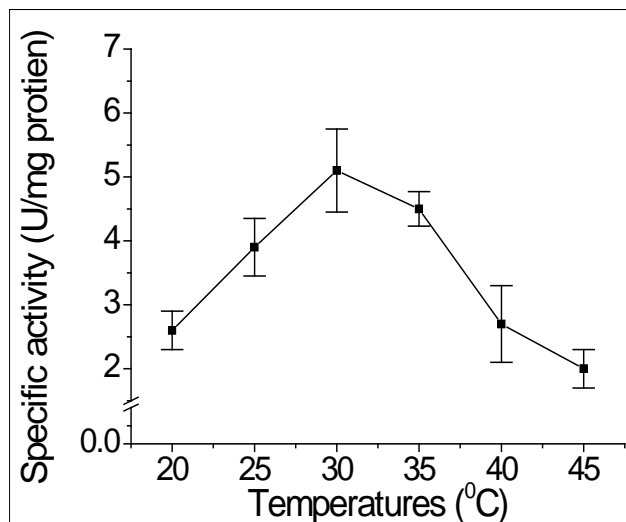
The effect of different incubation temperatures (20-45 °C) on chitinase activity was checked in liquid media. *B. subtilis* (A3) was used in present study. The activity of enzyme was increase dramatically with elevation of temperature up to maximum activity

5.1 U/mg at 30 °C but the activity of enzyme was reduced when the temperature increased (Fig. 3).

#### Effect of pH on enzyme activity

Fig. 4 shows the specific enzyme activity of chitinase produced by *B. subtilis* that incubated at different pH values in liquid media. The increase of pH stimulated the bacteria to produce the enzyme

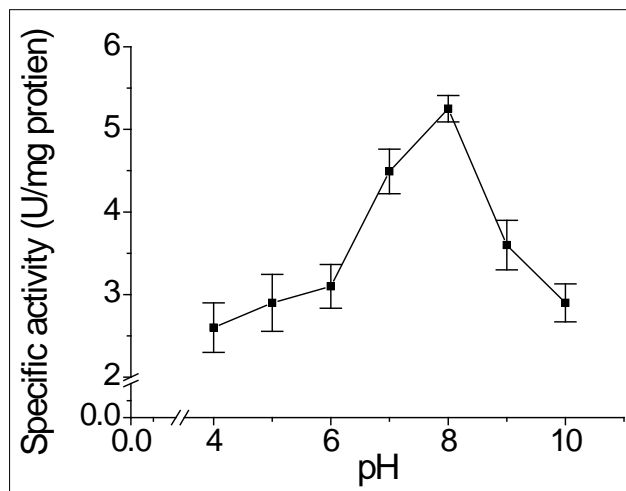
up to maximum enzyme activity (5.2 U/mg protein) at pH 8. But the enzyme activity decreased in pH higher than 8.



**Fig. 3** Effect of different temperature on specific enzyme activity of chitinase. The maximum enzyme activity was observed at temperature 30 °C.

## DISCUSSION

Chitinase is an enzyme capable of degrading chitin into chitooligomers and chitosan [9]. The chitinases play a direct role in biological control and clean the environment from several kinds of pollutants like insects and plants [10]. Chitinase can be extracted and purified from plant tissue, insects and microorganisms like fungus and bacteria [9,11].



**Fig. 4** Effect of different incubation pH on specific enzyme activity of chitinase. The maximum enzyme activity was observed at pH 8.

The chitinase characterized and purified in this study were extracted from the *B. subtilis* spp. isolated from soil. When grown in liquid media containing different nitrogen sources. The highest activity isolate that produced chitinase was *B. subtilis* (A3). The optimum chitinase activity of this isolate was reached after 4-5 days of incubation. The optimum temperature and pH of the chitinase was found at 30 °C and pH 8. In current study the partial technology of purification of enzymes was followed by using ammonium sulphate. The ion-exchange chromatography was used to get the high purified enzyme. In present study it was found adding casein improve the ability of bacteria to produce chitinase significantly as compared with other nitrogen sources. The chitinase optimum activity was variable according to the source of enzyme. Wang and Chang (1997) isolated two iso-enzymes of chitinase from *Pseudomonas aeruginosa* and they found that the optimum pH, optimum temperature, pH stability, and thermal stability of FI were pH 8, 50 °C, pH 6 to 9, and 50 °C; those of FII were pH 7, 40 °C, pH 5 to 10, and 60 °C [4]. An alkalophilic, chitinase-producing *Bacillus* sp. BG-11 was isolated which produced an extracellular chitinase. The purified chitinase exhibited a broad pH and temperature optima of 7.5-9.0 and 45 °C, 55 °C, respectively. The chitinase was stable between pH 6.0-9.0 and 50°C for more than 2 h [12]. Present study showed that the isolated chitinase is working in moderate low temperature and high pH but we did not clear the stability of this enzyme that is why further work is required before decide the possibility of using this enzyme in certain application. In can be concluded from present study that *B. subtilis* local isolate has a good ability to produce chitinase. The optimum condition of this enzyme is 30 °C and pH 8.

## Conflict of interest

The author declares that he has no conflict of interests.

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