



Production and Screening of Streptomyces-Extracellular Chitinase

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Abstract

The aim of this research was to produce *Streptomyces*-extracellular chitinase and screen its antifungal activity on a clinically isolated *Candida albicans*. The *Streptomyces* were isolated from an agricultural farmland; they were identified and screened for the chitinase production. Effects of time, temperature, pH and nitrogen sources on the chitinase production were determined using standard methods. Ammonium sulphate precipitation was used to partially purify the chitinase. Protein concentrations were determined spectrophotometrically using bovine serum albumin as standard. Agar-well diffusion method was used to evaluate the antifungal activity of the chitinase on *C. albicans*. The isolated *Streptomyces* were of three (3) strains, and all the strains are Gram positive, catalase positive, oxidase positive while, Strain A and C are indole positive and only Strain B is citrate positive. The maximum chitinase production was at 72 h, 40°C and when yeast extract was used as the nitrogen source. Ammonium sulphate (80%) precipitation yielded the highest enzyme activity of 39.0U/ml. The maximum enzyme activity was observed at temperature of 40°C, pH 5.5 and 1.0% colloidal chitin (substrate). The partially purified chitinase showed a zone of inhibition of 20.11 ± 1.26 mm against the *Candida albicans*. This result has no significant difference ($P > 0.05$) when compared with that of the standard drug (Fluconazole) with 21.42 ± 0.08 mm zone of inhibition. These findings suggest that *Streptomyces* at favourable conditions produce chitinase, and this enzyme can be used as an antifungal agent on *Candida albicans* and other chitin containing fungi.

Introduction

Antimicrobial resistance to medicines is now a rising concern to global public health that transcends country borders and socioeconomic divides. It affects people all over the world (Mitchell, 2019). Antimicrobial resistance has ramifications for human, animal, and ecological health. A multifaceted issue of crisis proportions has erupted, with major ramifications for the economy, health, and human well-being. A growing number of illnesses caused by bacteria, parasites, viruses, and fungi are being made more difficult to prevent and cure as a result of antimicrobial resistance (Suresh et al., 2018). Increased global travel, medical tourism, and trade, as well as a lack of access to effective drugs, the availability of substandard and falsified products, the misuse of antibiotics in food production, and a lack of effective infection control measures all contribute to the emergence and spread of antimicrobial resistance.

Streptomyces has produced a large number of bioactive chemicals with significant economic potential. In order to identify novel bioactive compounds, they are regularly tested. The capacity of *Streptomyces* to generate bioactive secondary metabolites such as antifungals, antivirals, anticancer agents, anti-hypertensive agents, and antibiotics and immunosuppressive agents is the most intriguing feature of this organism (Khan, 2011).

The production of chitinase by *Streptomyces* spp (Actinomycetes) as an antifungal has attracted attention due to its numerous applications in industries, including the control of phytopathogenic fungi, in the food industry by increasing the shelf-life of fruits, biopesticidal properties, the production of single cell protein, the production of oligosaccharides, and the production of medicines that can be used in clinical settings, among others. The annual global capitulate of chitin is implicitly estimated to be between 1 and 100 billion metric tons, making chitin the second most prevalent polysaccharide after glucose.

In addition to being an industrially significant enzyme, chitinase (EC 3.2.1.14) belongs to the glycosyl hydrolase family and degrades chitin (an insoluble linear -1, 4-linked polymer of N-acetylglucosamine) into its end products, which include N-acetylglucosamide and chitobiose, among other things. They are also responsible for stimulating the production of chitinase, which is an enzyme that breaks down chitin (Montgomery and Kirchman, 1994). Chitinolytic organisms account for about 90–99 percent of all microorganisms, with Actinomycetes accounting for the majority of them. As soil microorganisms, Actinomycetes, and especially *Streptomyces* species, are significant saprophytic soil microbes that are excellent sources of new antimicrobial agents, enzyme inhibitors, immunomodifiers, and vitamins (Kumar & Gupta, 2006). In most cases, the screening and isolation of microorganisms that are capable of generating chitinase takes place on a medium that contains chitin. As a result, the emphasis of this study was on the use of the separated chitinase from the *Streptomyces* fungus on clinically isolated fungal species.

Methods

Soil Sample

In January 2018, samples of soil were gathered from agricultural farmlands located at Anambra State University in Uli, Ihiala Local Government Area. The first step was to take off the top layer of soil and dig out a 30cm wide patch. Samples were obtained from Kebbi State University of Science and Technology, Aliero Biochemistry Laboratory and then transferred in sterile plastic bags to the lab for examination.

Culture Media

The culture media such as potato dextrose agar, sabouraud dextrose agar, colloidal chitin medium and chitin Yeast-extract salt medium were used. They were purchased from Drug field Pharmaceuticals LTD and they were of standard grade.

Chitin

Chitin was purchased from Drug field Pharmaceuticals LTD.

Standard Antifungal Drug

Fluconazole was used and was purchased from Drug field Pharmaceuticals LTD.

Candida albicans

Candida albicans was used to test the activity of the chitinase. It was obtained from Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria.

Preparation of Culture Media

Potato Dextrose Agar (PDA)

This was done by suspending 7.8g of potato dextrose agar in 200ml distilled water. It was heated on a hot plate and sterilized by autoclaving at 121°C for 15 minutes. It was mixed well and poured into sterile petri dishes.

Sabouraud Dextrose Agar (SDA)

This was done by suspending 6.2 grams of Sabouraud dextrose agar in 250mls of distilled water. It was heated to boiling which dissolved the medium completely and sterilized by autoclaving at 121°C for 15 minutes. It was cooled to 45-50°C and poured into sterile petri dishes.

Colloidal Chitin Medium

The process to make colloidal chitin was developed by Hsu and Lockwood, which will be detailed below (1975). For 20 grams of chitin powder, 250 ml of strong hydrochloric acid was used to dissolve it. Stirring occurred at intervals of 5 minutes for one hour at 4°C. To begin, the Chitin-Conc. HCl mixture was put into a 600ml container that was half-filled with cold water. While stirring, the mixture was poured into the container. The suspension was prepared with water containing chitin and was then increased in volume to 500ml by adding more water. To settle the chitin, the suspension was left to rest overnight, after which it was in a more concentrated state. To re-suspend the chitin, water was added while the supernatant liquid was being gently siphoned out. In order to get the acid fully disappear and get the pH down to 6-7, the hydrolyzed chitin was rinsed with distilled water approximately six times. The colloidal chitin was autoclaved for 15 minutes at 121°C and then was collected and filtered using Whatman filter paper. Colloidal chitin was kept at 4°C and obtained by collecting it.

Chitin Yeast-extract Salt (CYS) Medium

Two grams (2g) of colloidal chitin and 3g of agar were suspended into 200ml of distilled water, followed by addition of 0.25g yeast extract, (1g of K_2HPO_4 , 0.05g $F_2SO_4 \cdot 7H_2O$ and 0.5g $MgSO_4 \cdot 7H_2O$) then mixed together and heated to dissolve. The mixture was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile petri-plates.

Isolation of Streptomyces

One (1g) gram of soil sample was soaked with physiological saline (0.8 percent NaCl) and combined with one (1g) gram of calcium carbonate before being incubated at 28 degrees Celsius for thirty minutes (Limon et al., 1999). The incubated mixture was transferred to conical flasks holding 100ml of normal saline (0.85 percent NaCl), which were shaken together and left to stand for 15 minutes. The suspension part was serially diluted up to a concentration of 10^{-5} . (Kumar & Jadeja, 2016). PDA medium was infected with an aliquot of 0.1 ml of each dilution in order to test the results. Plates were incubated at 30 °C for 7 days, after which representative colonies were chosen and streaked onto fresh plates of PDA media, as described above. The subcultured *Streptomyces* species were kept on agar plates at 4 degrees Celsius until they were needed for further usage (Korn-Wendisch & Kutzner, 1991). Striking isolated bacterial colonies onto newly prepared agar plates allowed them to be sub-cultured, and from there, stock cultures of the isolated bacteria were grown on slants and kept at 4°C until needed (Hussein, 2013).

Characterization of the Bacterial Isolates (Streptomyces)

Some tests such as catalase, gram staining, citrate, oxidase and indole tests were carried out to confirm the characteristic features of the isolates as described by Oyeleke & Jibrin, (2009).

Catalase Test

Colonies were picked using sterile wire loop and placed on a centre of clean, grease-free glass slides. Three (3) drops of hydrogen peroxide were added. The presence of bubbles showed a positive test while absence indicated a negative test.

Oxidase Test:

A smear was prepared from the culture and flooded with three drops of 1% tetramethyl-p-phenylen-diamine-dihydrochloride solution (oxidase reagent) and mixed together. A positive reaction was indicated by change of colour from green to blue.

Gram's Staining

A subculture was picked from a plate after the wire loop had been heated under a spirit lamp flame and allowed to cool, a small smear was created by mixing the colony in a drop of normal saline, and the smear was allowed to air dry, was slightly flame-fixed with a spirit lamp, and was stained with crystal violet for two minutes before being washed with distilled water. The slide was then given a Lugo iodine bath for a minute and after being rinsed with distilled water, it was put in acetone to remove the color. Afterwards, it was washed again and allowed to soak in safaramine. We next inspected it with the oil immersion objective lens after air-drying it and re-wetting it many times with distilled water.

Indole Test

Organisms were incubated in nutritional broth for 24 hours, and then they were cultured in 5ml of the soup. Kowac's indole reagent was applied to the sample after it had been incubated (eight drops). It was shook up and then shaken. A red color appearing in the reagent layer in the first minute was a sign of a good response. Yellow stains were produced on negative findings.

Citrate Test

Simmons citrate agar was inoculated with the isolate, using sterile wire loop and the plate was incubated for 24-48hours at 37°C. A deep blue color development indicated a positive result.

Screening of the Chitinase Producing *Streptomyces*

A plate assay technique was used to test soil bacteria for chitinase production after a serial dilution and physical treatment was used to isolate several species of *Streptomyces* (El-Tarabily et al., 2000). The entity was introduced into a sterile chitin-yeast extract media. The experiment was performed using 0.1% Congo red solution, which was poured over the plates and allowed to incubate for three days before clearing. After identifying the organisms that displayed the zone of clearing, scientists cultured these organisms on sterile starch casein agar slants kept at 4°C.

Chitinase Production

The medium Brzezinska et al. (2014) developed to be utilized for chitinase synthesis by *Streptomyces* (Strain A) was used, except for some minor changes. Colloidal chitin, a protein-like substance used to imitate chitin, was dissolved in 1 percent vinegar. In order to regulate the pH to 6.0, we added (g/L) yeast extract, K₂HPO₄, MgSO₄·7H₂O, NaCl₂, and water. The resulting solution was called "the medium" and consisted of (g/L) yeast extract, K₂HPO₄, MgSO₄·7H₂O, NaCl₂, and 1 percent colloidal chitin dissolved in water, with a pH of 6.0. To start the experiment, the scientists made 100 ml of the media recipe and then added 1 ml of the spore suspension. To make the manufacturing happen, it took 4 days at 35°C in an incubator.

After a three-day incubation, the cultures were taken out, and the centrifuging process began at 10,000rpm and 10 minutes. for subsequent testing, the culture supernatant served as enzyme (chitinase) source (Brzezinska et al., 2014).

Optimization of Chitinase Production

Incubation Time

Further optimization was performed to fine-tune the outcome of varying operating factors on chitinase production. Spore suspension was poured into the incubation medium, which was then incubated at 35°C with the incubator shaking every so often. To find the ideal incubation time for chitinase production, the cultures were harvested every 24 hours for 96 hours, spun at 10,000rpm for 15 minutes, and the supernatant was tested for chitinase concentration. Then, the cultures were centrifuged at 10,000rpm for 15 minutes and the supernatant was tested for chitinase concentration and protein concentration.

Effect of Temperature

To determine the best incubation temperature, the chitinase production medium was produced in five flasks and then, during a 96-hour incubation period, the production of chitinase was monitored after holding the media at a range of temperatures (25 to 60°C) for a 24-hour period.

Effect of pH

The optimal pH for chitinase synthesis was determined by changing the pH of the production medium from 2.0 to 9.0 while maintaining the optimal temperature and incubation time for the enzyme production. During the 96-hour period, the cultures were collected every 24 hours and centrifuged at 10,000rpm for 15 minutes, after which the supernatant was submitted to the chitinase test and the protein content was measured.

Nitrogen Source

The effect of various sources of nitrogen on chitinase production was assessed by incubating 3 ml of inoculum in 50 ml of sterile production medium for 96 hours at 37 C with 100 rpm shaking at 100 rpm with a different nitrogen source, including yeast extract, beef extract, peptone, ammonium sulphate, and L-glutamine. The enzyme activity and protein content were evaluated by measuring the culture filtrate that was taken every 24 hours.

Protein Content Determination

In accordance with Bradford (1976), the protein content in filtrates was measured using a spectrophotometer (at 595 nm) and bovine serum albumin as a standard, as described in Bradford (1976).

An initial series of standard dilutions with 0.15M NaCl was carried out to the final concentrations of 0 (blank= no protein), 250g/ml, 500g/ml, 750g/ml, and 1500g/ml. A second series of serial dilutions of the unknown material (filtrate) was carried out as well. Every one of the aforementioned solutions was put to a separate test tube along with 0.5% Coomassie Blue, which was mixed by inversion. A blank was used to calibrate the spectrophotometer after the mixtures were mixed. The absorbance of the standard and the samples was measured at 595nm in 5 minutes.

Partial Purification of Chitinase Using Ammonium Sulphate

In this experiment, about 80mL of culture filtrate solution was treated with 70% saturated ammonium sulphate solution. Overnight at 4 degrees Celsius, the crude enzyme was constantly agitated with a magnetic stirrer and centrifuged at 10,000 rpm for 15 minutes, after which the

supernatant was discarded and the pellets were collected with 0.02 mL of 0.02 M Triacetate pH 8.8 and dialyzed with the same buffer.

Chitinase Activity Assay

In accordance with the literature, colloidal chitin was utilized as a substrate (Choi et al., 1997). 3ml of 1 percent colloidal chitin in acetate buffer (50Mm, pH 6.0) was used in the experiment. Inoculated at 30oC for 30 minutes with a 1ml of enzyme, the mixture was then discarded. The hydrolysis process was brought to a close by the addition of 0.6mL of dinitrosacylic acid (DNS). The mixture was placed in a boiling water bath for 15 minutes, then cooled before being centrifuged to remove the insoluble chitin residue. The resultant adduct was measured at 540nm in a UV double beam spectrophotometer using an ultraviolet laser. Once an enzyme activity unit had been established as the quantity of enzyme that was catalyzing the release of one molecular weight of N-acetyl D-glucosamine per milliliter of solution per minute, the amount of enzyme was divided by two.

Optimum pH of Chitinase

In a reaction mixture containing 1 percent colloidal chitin as substrate and 20mM of the partially purified enzyme (0.502g), the optimal pH of the partially purified chitinase activity was determined by mixing the enzyme with various pH buffers, including acetate buffer (pH 4.0–6.0), phosphate buffer (pH 6.0–8.0), Tris-HCl buffer (pH 7.5–9.0), and glycine buffer (pH 9.0–12). In order to halt the process, the mixture was incubated for 1 hour at 37oC and then boiled for 5 minutes (Ruiz-Sannchez et al., 2005). After chilling, the mixture was centrifuged, and the quantity of reducing sugar in the supernatant was measured using a modified DNS technique, which was previously described. The optimal pH values that were acquired were utilized to determine the optimal temperatures for the experiments.

Optimum Temperature of Chitinase

In order to find the optimal temperature for the chitinase activity, enzymatic reactions were carried out at different temperatures (25, 30, 40, 50, and 60 oC) using colloidal chitin to determine the optimal temperature. 50mM Tris-HCl buffer and 0.502g of enzyme are used (pH 5.5). The mixture was incubated at 37oC for 1 hour before being brought to a boil for 5 minutes to bring the reaction to a halt. Once the mixture had cooled, it was centrifuged, and the quantity of reducing sugar in the supernatant was measured using a modified DNS technique (Ruiz-Sannchez et al., 2005).

Effect of Substrate Concentration

In order to determine the substrate concentration, the medium was produced with various substrate (chitin) concentrations in varied concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 percent). Shaking was used to keep them alive throughout incubation. The mixture was incubated at 37oC for 1 hour before being brought to a boil for 5 minutes to bring the reaction to a halt. Once the mixture had cooled, it was centrifuged, and the quantity of reducing sugar in the supernatant was measured using a modified DNS technique (Ruiz-Sannchez et al., 2005).

Antifungal Performance of the Chitinase on *Candida albicans*

The antifungal activity of chitinase was evaluated using the agar well diffusion technique, which was developed by Zhang et al., in this study (2013). It was decided to utilize Sabouraud dextrose agar as the culture medium for this test. Pre-sterilized petri dishes (25 mL each) were used to distribute the molten sabouraud dextrose agar, which was allowed to cool before using. The fungus strain was seeded onto the agar plates in this experiment. The plates were allowed

to harden for a period of time. With the use of a cork borer, wells of 6 mm in diameter were punched into the agar after it had solidified. For each plate, three wells were created in advance. Two of these three wells were filled with 0.2 ml of the crude culture filtrate and 0.2 ml of partly purified chitinase, respectively, while the third well was left empty. The third well was filled with 0.2 mL of a typical antibiotic medication (Fluconazole). The Petri plates were incubated for 72 hours at 37 degrees Celsius. It was next necessary to measure the diameter of the inhibition zone that had developed around each well after incubation and record the results in millimeters (mm). The tests were carried out in triplicates to ensure accuracy.

Statistical Analysis

Some of the data obtained were subjected to statistical analysis using Microsoft Excel (2013 version).

Results and Discussion

Morphological Characteristics of the Isolated *Streptomyces*

Plate 1 shows the various *Streptomyces* strains isolated from the soil sample. Plate 1A sample appeared milky in colour, with a spiral spore chain, the spore surface was smooth and the odour was earthy. The *Streptomyces* isolated in Plate 1B was white in colour, with a spiral spore chain which was smooth on the surface and had an earthy odour. The *Streptomyces* isolated in Plate 1C was grey in colour, with a spiral spore chain and smooth surface and had an earthy odour.

Biochemical Characteristics of the Isolated *Streptomyces*.

The biochemical characteristics of the isolated *Streptomyces* were determined and presented in Table 1. All the samples were Gram positive, catalase positive, oxidase positive while Strains A and C are indole positive and only Strain B is citrate positive.

Screening of the isolates for the Production of Chitinase Enzymes

Plate 3 of assay fed with Congo red solution after incubation period, showing the chitinase production of *Streptomyces* spp using 1% colloidal chitin containing medium and formation of clear zone of hydrolysis. Plate 2A and 2B shows clear zone of hydrolysis on three spots while Plate 2C shows no zone of hydrolysis.

Optimisation of Chitinase Production from the Bacteria Isolate

Time of Maximum Chitinase Production

Figure 1 shows the effect of time on chitinase production. After 24 h of incubation, chitinase production increased progressively through 48 h and reached maximum levels at 72 h. Chitinase production was found to decline as the incubation period further extended. As the biomass increased, the protein concentration increased. The maximum chitinase production (protein concentration) was at 8mg/ml while that of biomass was at 117mg/ml.

Figure 2 shows that as the protein (chitinase) concentration increased, the enzyme activity increased. At protein concentration of 8mg/ml, the enzyme activity was 12 U/ml.

Effect of Temperature on Chitinase Production

The effect of temperature on chitinase production is presented in Figure 3. At 30 °C and 40 °C, the chitinase production was 14.0mg/ml which was the maximum temperature for which chitinase was produced while at 25 °C, 50 °C and 60 °C chitinase production was extremely minimal. Figure 4 shows that at 72 h of incubation, the enzyme activity was maximum at 14.0

mg/ml. After 40 °C, production gradually decreases as increase in temperature based on chitinase activity assay.

Effect of Supplemental Nitrogen Sources on the Chitinase Production

The effect of different nitrogen sources (yeast extract, peptone, beef extract, ammonium sulphate and L-Glutamine) on chitinase production on the protein (chitinase) concentration is presented in Figure 3. It was observed that the production medium containing Yeast extract showed the maximum chitinase concentration of 13.50 mg/ml at 72 h while ammonium sulphate extract showed the minimum chitinase concentration of 5.0 mg/ml at 72 h. Hence, production medium containing Yeast extract showed a maximum chitinase activity of 13.07 U/ml at 72 h while ammonium sulphate extract showed a minimum chitinase activity of 4.60 U/ml at that 72 h in Figure 6.

Partial purification of the Chitinase by Ammonium Sulphate Precipitation

The purification of chitinase using ammonium sulphate precipitation is presented in Figure 7. As the ammonium sulphate concentration increased from crude mixture (0%) to 80%, the enzyme activity also increased. The ammonium sulphate concentration of 80% yielded the highest enzyme activity of 39.0 U/ml while the filtrate yielded the lowest enzyme activity of 12.10 U/ml.

Optimum Temperature for Chitinase Activity

The effect of temperature on chitinase activity showed that from 25 °C, the enzyme activity increased progressively as the temperature increased, reaching the maximum activity of 76 U/ml at 40 °C. After 40 °C, the enzyme activity started decreasing until minimal activity of 1.00 U/ml was observed at 60 °C (Figure 8). Hence, the optimum temperature at which chitinase activity is maximum was 40°C.

Optimum pH for Chitinase Activity

Figure 9 shows the effect of pH on chitinase activity. In the acidic pH of 2.0 and pH 3.0, the chitinase activity was minimal. As the pH increased, the chitinase activity also increased to the peak level at pH 5.5 with enzyme activity of 60.0 U/ml. After this pH, the chitinase activity decreased as the pH increased to 7.0. A switch to the alkaline pH showed a sharp decrease in the chitinase activity, which became 0U/ml.

Effect of Substrate Concentration on the Chitinase Activity

As the substrate (colloidal chitin) concentration increased, chitinase activity also increased before subsequently decreasing as the substrate was increased. Among the five different concentrations of colloidal chitin tested, colloidal chitin at 1.0% considerably increased the chitinase activity to 79.8 U/ml followed by 1.5% which gave 50.50 U/ml chitinase activity. Beyond 1.0%, increase in substrate concentration decreased enzyme activity (Figure 10).

Antifungal Performance of the Partially Purified Chitinase on *Candida albicans*

The antifungal activity of the *Streptomyces*-Extracellular Chitinase on the *Candida albicans* is presented in Table 2. The partially purified chitinase showed a higher zone of inhibition (20.11 mm) than the crude filtrate (12.42 mm). Fluconazole which was used as the standard drug showed the highest activity (21.42 mm).



Plate 1. *Streptomyces* isolated from the soil sample, Strain A (left), Strain B (middle) and Strain C (right).



Plate 2. *Streptomyces* subculture. Strain A - milky (left), Strain B - white (middle) and Strain C - grey (right).

Table 1. Biochemical Characteristics of the Isolated *Streptomyces*

Test	Observation		
	Strain A	Strain B	Strain C
Gram's staining	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
Indole	+	-	+
Citrate	-	+	-

+ = Positive, - = Negative

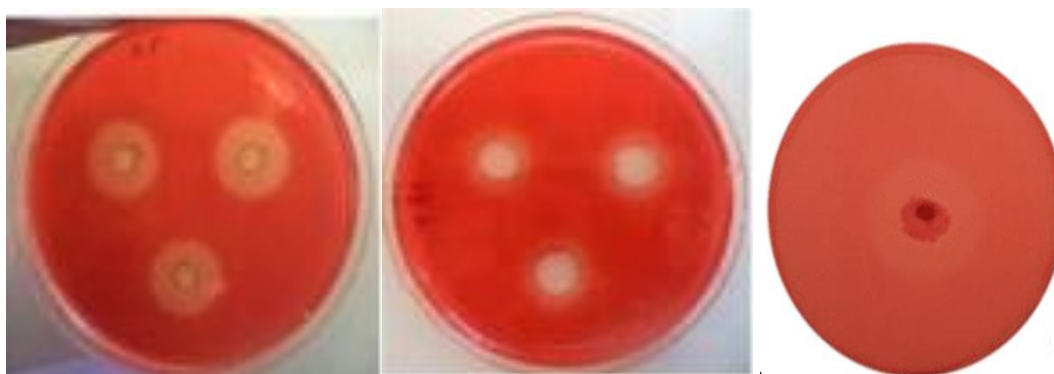


Plate 3. *Streptomyces* culture showing clear zone for chitin hydrolysis by the chitinase. Strain A (left), Strain B (middle) and Strain C (right).

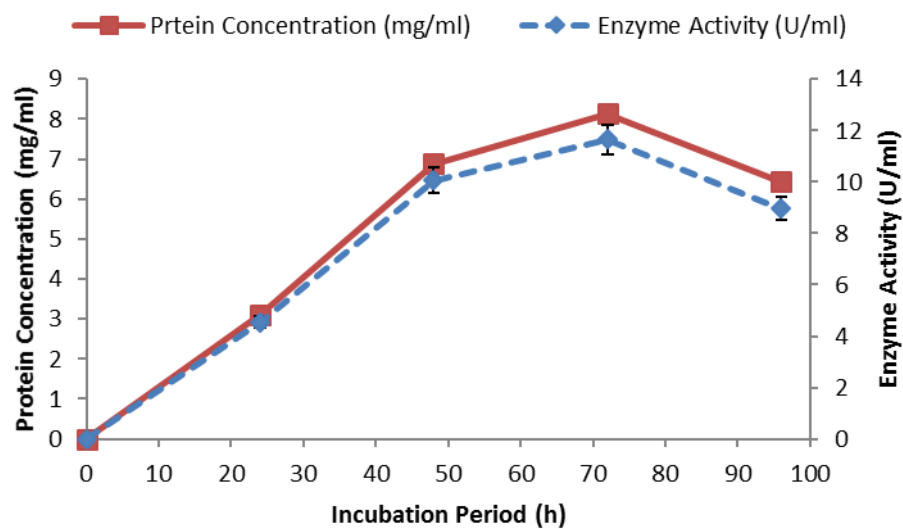


Figure 1. Time of Maximum Chitinase Production and Enzyme Activity

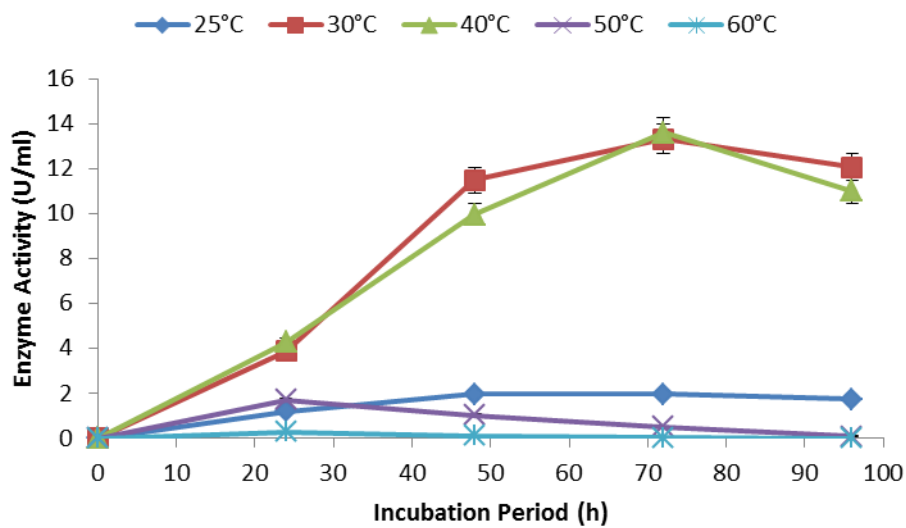


Figure 2. Effect of temperature on Chitinase Activity

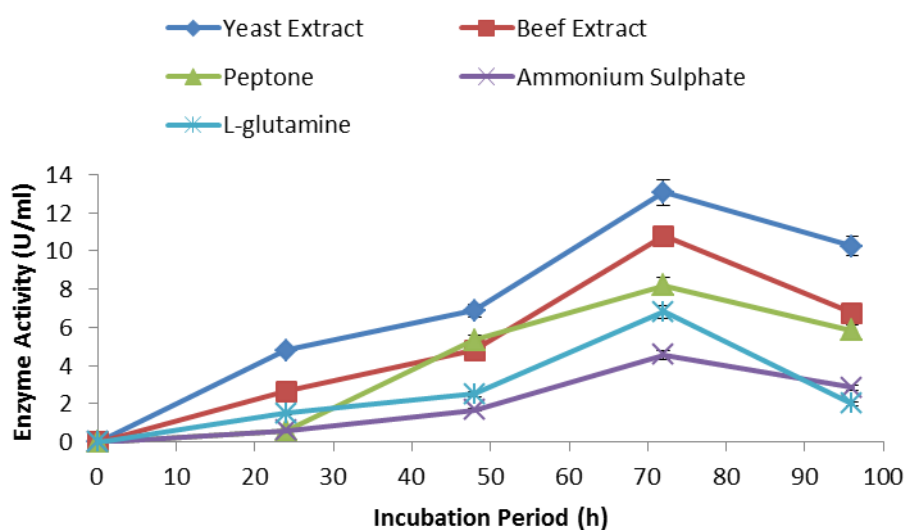


Figure 3. Effect of Supplemental Nitrogen Sources on the Chitinase Activity

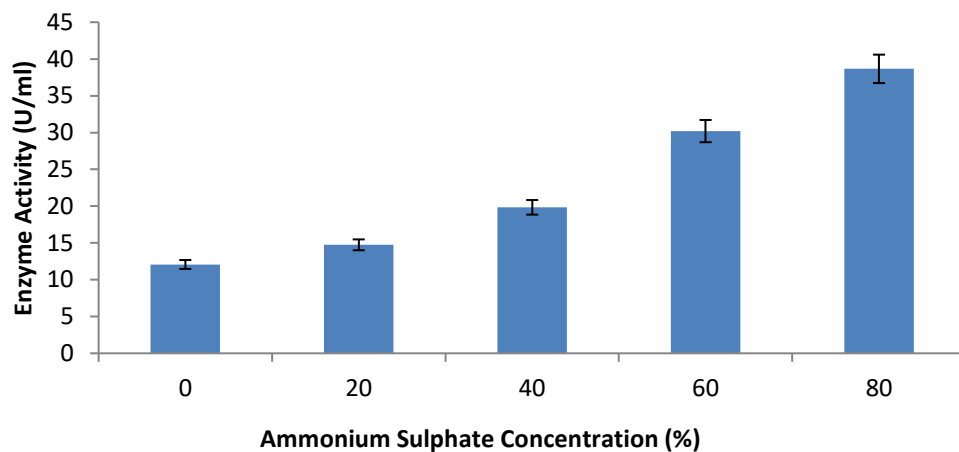


Figure 4. Partial purification of the Chitinase by Ammonium Sulphate Precipitation

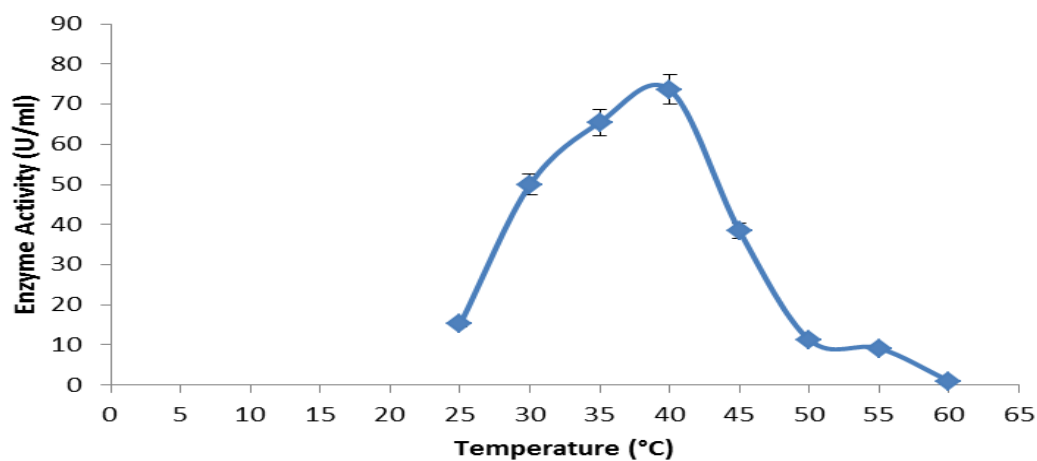


Figure 5. Optimum Temperature for Chitinase Activity

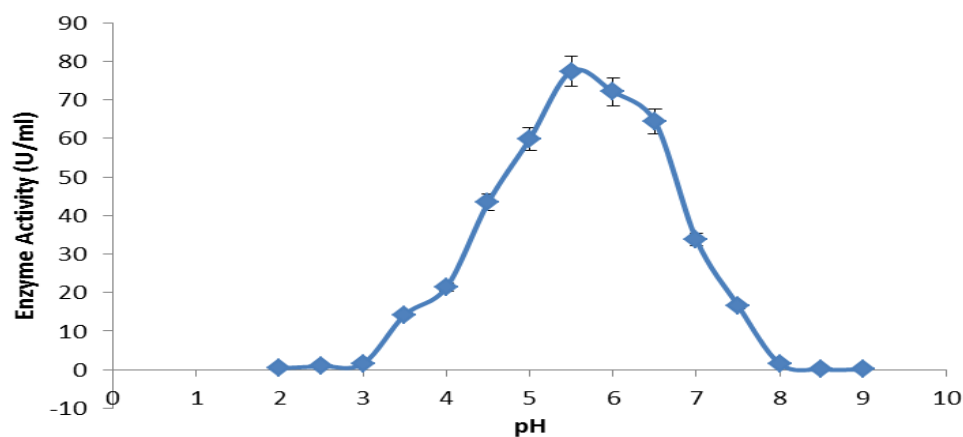


Figure 6. Optimum pH for Chitinase Activity

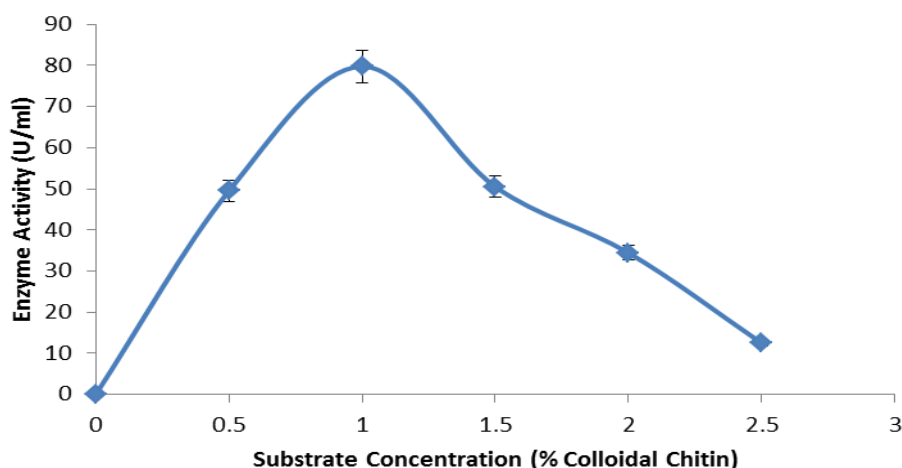


Figure 7. Effect of Substrate Concentration on the Chitinase Activity

Table 2. Antifungal Activity of *Streptomyces*-Extracellular Chitinase on *Candida albicans*

Test Material	Zone of Inhibition (mm)
Crude Culture Filtrate (13.5mg/ml)	12.42 ± 0.78
Partially Purified Chitinase (0.5mg/ml)	20.11 ± 1.26
Fluconazole (10 mg/ml)	21.42 ± 0.08

Values are presented as Mean ± Standard Error of Mean of triplicates.

Organisms are the main decomposers of organic waste, but they also perform a variety of other functions, including as providing nitrogen to developing plants via nitrogen fixation, detoxifying toxic compounds, and producing products that may promote plant development (Sowmya et al., 2012). This study investigated the biochemical properties of the isolated *Streptomyces* strains. The bacterial isolates were all Gram positive, catalase positive, and oxidase positive, with the exception of Strain A and C, which are both indole positive and only Strain B being citrate positive (Figure 1). Various chitinolytic enzymes have been discovered in the genomes of several *Streptomyces* species (Narayana & Vijayalakshmi, 2009). Chitinase has gotten a lot of interest lately because of its potential application as a biocontrol agent as well as for the development of transgenic plants (Narayana & Vijayalakshmi, 2009).

The impact of incubation time on the synthesis of chitinase was studied by monitoring the enzyme production on solid medium for a period ranging from 24 to 96 hours, respectively. After 72 hours of incubation, the highest level of chitinase synthesis was observed. It was reported by Nawani & Kapadnis (2004) that the synthesis of chitinase by *Streptomyces* sp. NK1057 was high after 120 hours of incubation, which was much longer than the 72 hours observed in our study. Using *Streptomyces halstedii* as the model organism, Joo (2005) discovered that the highest production of chitinase was obtained after 72 hours of incubation, which is consistent with the results of this study.

The temperature of the culture medium has a significant impact on the synthesis of chitinase in bacteria. The optimal temperature for chitinase synthesis, which was determined to be 40 degrees Celsius in the current study, is in accordance with previous studies conducted by Wang et al. (1996) and Lu et al. (1996). (2002). In contrast, according to a study conducted by Sowmya et al. (2012), the optimum temperature for chitinase production in *Trichoderma*

harzianum was 28 degrees Celsius, and *Serratia marcescens* produced a significant amount of chitinase at a controlled temperature of 30 degrees Celsius in continuous culture (Figure 1).

The majority of bacteria have been shown to generate their highest amounts of chitinase at neutral or slightly acidic pH, while fungi have been found to secrete their highest levels of chitinase at acidic pH. (Gomaa, 2012). Although the greatest enzyme activity was found at pH 5.5 in this study, this value was lower than that reported by several other studies in the same field. The optimal pH and temperature for chitinase synthesis in *Microbispora* sp. V2 have been found to be 7 and 40 degrees Celsius, respectively (Nawani, 2002). It was discovered by Taechowisan et al. (2003) that the synthesis of chitinase by *S. aureofaciens* was most efficient at pH 6.5-7.

The availability of nitrogen in the culture medium is critical for the development of microorganisms as well as their ability to produce metabolites. The greatest level of chitinase synthesis was achieved using yeast extract, which was the most abundant of the five (5) nitrogen sources tested. Another important element is the concentration of colloidal chitin, which has been shown to stimulate the synthesis of chitinase in a variety of bacteria (Shanmugaiah et al., 2008). According to Limón et al. (1999), when N-acetyl glucosamine and glucose are added to the medium of *Trichoderma harzianum*, a modest increase in chitinase synthesis is seen in the bacteria. In contrast to previous reports by Rattanakit et al., (2002) and Al-Ahmadi et al., (2008), the results of this study showed that, among the nitrogen sources added to the basal medium, ammonium sulfate was the most effective in increasing the amount of chitinase production by *Aspergillus* spp. SI-13, whereas peptone, yeast extract, and urea had little effect on the amount of chi

The highest enzyme activity was seen in the culture media that had been supplemented with 1 percent colloidal chitin. As previously reported by Thompson et al. (2001), the production of chitinase from *Staphylococcus aureofaciens* was optimized with a colloidal-chitin concentration of 1 percent. This result was consistent with their findings, which stated that the optimal concentration of colloidal chitin was 1 percent. It was discovered by Narayana & Vijayalakshmi (2009) that the strain *Streptomyces* sp. ANU 6277 generated the highest amounts of chitinase in culture medium modified with 1 percent chitin, and that this medium was determined to be appropriate for the highest possible production of the enzyme.

In this study, both the crude chitinase culture and the partially purified chitinase inhibited the growth of the tested fungus (*Candida albicans*), suggesting that chitinase has the potential to be used as an antifungal agent. Furthermore, the partially purified chitinase showed a greater zone of inhibition than the crude culture, suggesting that the chitinase has the potential to be used as an anti Palmer & Freeman (2004) reported that they isolated eight predominant actinomycetes strains from marine soil samples collected from the Nizampatnam coast of Andhra Pradesh, and that all of them exhibited high chitinase activity on colloidal chitin agar medium with an average clearing zone of 20mm, suggesting the potential use of chitinases as biocontrol agents. Hoster et al., (2005) discovered that out of thirteen distinct chitin-degrading bacterial strains that were isolated from soil and sediment samples, five of these strains (SGE2, SGE4, SSL3, MG1 and MG3) showed antifungal activity against phytopathogenic fungi. It was discovered via sequence analysis that ChlS of MG3 is very similar to chitinase from *Streptomyces* species, which has been shown to have considerable antifungal action. The current results are consistent with a previous study in which chitinases from *S. griseus* were shown to be active against fungus such as *Aspergillus* spp. and *Trichoderma reesei*, among other species (Hiroshi et al., 1994). The antifungal activity of isolated chitinase from *Streptomyces* sp. M-20 against *Botrytis cinerea* was determined by utilizing an agar diffusion

test on the bacteria. Chitinases from *Streptomyces* have been shown to have a variety of antifungal actions, including the suppression of spore germination, the elongation of germ tubes, and the bursting of spores (Narayana & Vijayalakshmi, 2009).

Joo, (2005) investigated the antifungal activity of pure and crude chitinases generated by *Streptomyces halstedii* in the presence and absence of fungicides. Pure and crude chitinolytic enzymes both suppressed the development of phytopathogens to a lesser or greater degree, depending on their concentration. *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, and *Stemphylium lycopersici* all had their mycelium growth suppressed by pure chitinases, while unpurified chitinases were also inhibited by *Phytophthora capsici*, which is a chitinase that has not been purified (Brameld et al., 1998).

Conclusion

The effect of temperature and supplemental nitrogen sources on chitinase production established the fact that these variables have a great influence on production and activity of chitinase. The fungal growth inhibition of *Candida albicans* in which the partially purified chitinase showed a higher zone of inhibition than the crude culture filtrate indicates that the purer the enzyme, the higher the activity. This has revealed the potentiality of chitinase to be a good candidate for antifungal activity.

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