Degradation of Chitin using Chitinase Produced from Molecular Identified Bacteria

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Abstract

Chitin and chitinolytic are beneficial enzymes for their biotechnological applications. Chitinases contribute to the generation of carbon and nitrogen in the ecosystem and can prohibit many fungal ailments that can threaten crop production worldwide. The goal of this work was the production and characterization of chitinase enzymes from bacteria isolated from the western region, Saudi Arabia for biocontrol fungal pathogens. Colloidal chitin from shrimp shells was prepared and used for isolation of chitinolytic bacteria on Mineral chitin agar medium from different sources. The most active isolates were AMM1 which was characterized and identified as *Alcaligenes aquatilis* using 16SrRNA. In conclusion, purified chitinase was success to produce from *Alcaligenes aquatilis* for medical uses and biocontrol process.

Introduction

Chitin is one of the major naturally occurring polysaccharides, found in fungi and exoskeleton of many arthropods, including insects (Gooday, 1990, Morganti, 2012, *et al.*, 2020). Chitinases of *Bacillus thuringiensis*: Phylogeny, Modular Structure, and Applied Potentials. Front in Microbiolgy. Vol. (10): p. 1-15.

Chitin and its related materials are widely utilized in wound healing, drug delivery, dietary fiber, and treatment of contaminated water (Muzzarelli, 1999). Chitinases are members of glycosyl hydrolases, that function in chitin hydrolysis to its monomer N-acetyl glucosamine via breaking the glycosidic bonds. Promoting the production of chitinase via various chitin compounds is a feature of specific enzyme-outputting strain. Many investigations utilize colloidal chitin to produce chitinase (Mane and Deshmukh, 2009, Hosny *et al.*, 2010) by some microorganisms (Wang *et al.*, 2002b, Chang *et al.*, 2010).

Many bacteria can yield chitinases, namely *Streptomyces*, (Blaak and Schremp, 1995) *Alteromonas* (Tsujibo, *et al.*, 1993) *Escherichia*, (West and Colwell, 1984) *Aeromonas* (Sitrit *et al.*, 1995). These bacteria have been separated from soil, shellfish waste and hot springs (Yuli *et al.*, 2004). *Aeromonas schubertii* was obtained from the soil of the south part of Taiwan utilizing a medium of colloidal chitin (Guo *et al.*, 2004). *Craniella australiensis* can give chitinase with potential antifungal versus *Aspergillus niger* and *Candida albicans* (Han *et al.*, 2009).

*Serratia marcescens* and *S. plymuthica* have a chiA gene, thus can be applied for bio management of phytopathology related to phytopathogenic fungi (Downing, 2000). Since most bacteria, that can produce chitinases, have been characterized and classified into groups A, B and C. Group A is the major type in nature (Metcalfe *et al.*, 2002).

Chitinase, with molecular weight 59kDa, has been obtained from 12 isolates of *Serratia marcescens*, from lettuce and spinach, utilizing DEAE Sephadex ion-exchange chromatography and Sephadex G-200 gel filtration. The pure enzyme showed an optimum temperature of 50°C and a pH of 6.0. The enzyme is steady up to 50°C at pH 5-7 and can be stimulated by Ca$^{+2}$, Cu$^{+2}$, Mg$^{+2}$ and suppressed by Hg$^{+2}$.
Chitinase has a strong suppressing influence on versus *Fusarium solaini* (Zeki and Muslim, 2017). The molecular weights of chitinase range between 20 -o 80 kDa with optimum pH 5 - 8 and temperature of 40°C, (Brzezinska et al., 2014).

This study aimed to biocontrol of some fungal pathogens by the purified bacterial chitinase.

**Materials And Methods**

**Chitin and colloidal chitin preparation:**

The exoskeleton of the shrimp shells was collected from the Jeddah fish market, Kingdom of Saudi Arabia and cleaned repeatedly, colloidal chitin was prepared as described by Trachuk et al. (1996).

**Isolation and screening of bacteria for chitinase:**

Different samples, soil, plant leaves, marine soil, marine water, dung, and chitinous wastes (fish, crab and shrimp shell) were collecting from Jeddah, Kingdom of Saudi Arabia. Segregation and examination of the chitinolytic bacteria were carried out by lamination of the specimens on mineralized chitin agar medium (Kim et al., 2003) under incubation at 37°C for 5 days for obtaining the bacterial colonies. Bacteria were screened on nutrient agar until pure colonies were observed, then preserved using agar slants at 4°C until used.

**Chitinase assay:**

The activity of chitinase was estimated spectrophotometrically by measuring the quantity of free reducing function groups created post hydrolysis of colloidal chitin (Trachuk et al. 1996). The reaction admixture contains 0.5 ml colloidal chitin (1%) suspended in a buffer of sodium acetate (0.1 M, pH 5.5) and 0.3 ml of enzyme solution. After 10 min of incubation at 37°C (Memmert Incubator), 0.75 ml of dinitrosalicylic acid reagent (DNSA) was to pause the reaction (Joshi et al., 1989). The suspension was boiled in a water bath for ten min and centrifuged for 10 min at 9000 rpm (Sigma Laboratory Centrifuges 4K15). The optical density was recorded at 530nm using a UV spectrophotometer (UV-1650PC Spectrophotometric, SHIMADZO). The enzyme activity was estimated using a standard curve of various concentrations of *N*-acetylglucosamine (Sigma). The unit of enzyme activity is represented by 1μM of *N*-acetylglucosamine produced / min at 37°C (Trachuk et al. 1996).

**Studying the ability of selected isolates to produce the chitinase enzyme on the solid medium and Decomposition chitin:**

The bacterial isolates AMM1, AMM2, AMM8, AMM31 and AMM37 were selected as the best isolates for chitinases production. The isolates were coated on plates containing mineral chitin agar medium (A), then incubated 5 days at 35 °C. The ability of isolates to decompose the chitin can be detected by the appearance of a clear zone surrounding the growth.
Identification of the selected isolates of bacteria:

The most active bacterial isolates were chosen to identify the genus level by morphological and physiological studies (Holt *et al.*, 1994) in addition to 16SrRNA (Aly *et al.*, 2011).

Biocontrol of some fungi using the selected bacterium:

Six fungal isolates were obtained from the Microbiology lab. Biocontrol of some fungal using the selected bacterial isolate AMM1 was studied. Different agar media (Nutrient, Starch nitrate, malt extract, Mueller- Hinton, Sabouraud dextrose, Potato dextrose, Glucose- yeast extract and Czapek-Dox) and different fungi (*Trichophyton mentagrophytes, Microsporum gallina, Candida albicans, Fusarium oxysporum, Trichoderma harzianum, Aspergillus fumigatus*) were used. After the preparation of the agar media, the agar plates were inoculated from one side with the studied bacterium, then all plates were incubated at 30 ± 2 °C. After two days, fungi were inoculated perpendicular line and incubation was carried out at 28°C. After 3 days, the inhibition effect was determined (El-Tarabily *et al.*, 1997).

Enzyme purification

Chitinase was produced by the selected bacterium using the optimal conditions where the selected bacterium was allowed to grow in the best medium and under all previous studied optimal conditions. At the finish of the incubation time, the cell extract was collected by centrifugation at 5500 rpm for a quarter-hour at 4°C. Enzyme activity and protein concentration were estimated and then the cells were kept at 4°Ci as a raw chitinase enzyme till utilized. The enzyme was purified utilizing Sephadex G 100 and DEAE cellulose column chromatography (Tork *et al.*, 2018). After obtaining the pure enzyme, the characters of the enzyme were studied, including ideal temperature, pH and heavy metal effect (Aly *et al.*, 2011).

Determination of protein content:

The content of the total protein of cell-free supernatant and purified samples were assayed by the method of Lowary (1956) utilizing the standard curve of bovine serum albumin (BSA).

SDS-PAGE protein Electrophoresis and determination of molecular weight:

The clarity of the separated protein was evaluated by the SDS-PAGE on 10percentage gel (Laemmli 1973). This assay was carried out to measure the molecular weight of the purified Chitinase enzyme. Enzyme molecular weight was measured by standard protein with different low molecular weights (14-60 kDa)

Properties of the purified Chitinase:

The pure enzyme was incubated at various temperatures (30, 35, 40, 45, 50, 55 and 60°C) to study the ideal temperature. Post incubation, at those temperature degrees, the tubes containing the incubation admixture were chilled and estimated for chitinase activity as shown before.
For studying the ideal pH, the reaction admixture was incubated at various pH (7.0, 7.5, 8.0, 8.5, 9.0 and 10.0) utilizing various buffers for a half-hour. Post incubation time at 45°C, the tubes were assayed for Chitinase activity as previously mentioned. Also, the substrate (chitin) was added into the reaction admixture at various concentrations (1-8 mg/ml of borate buffer, pH 8.0 and incubation temperature of 45°C for a half-hour. To estimate the ideal enzyme concentration, the purified enzyme was added to the reaction mixture at different concentrations (25, 50, 75, 100, 125 and 150 μl) (0.2-1 U/ml), then incubated at 45°C, at pH 8 for a half-hour. All these ions and chemicals (Ca²⁺, Zn²⁺, Cu²⁺, Fe²⁺, Co²⁺, Hg²⁺, Fe²⁺, EDTA), were applied to the reaction admixture at a concentration of 10⁻³ M, the pH was justified to 8.0 at 45°C for a half-hour.

**Effect of a purified enzyme on spore germination on water agar medium:**

In this experiment, enzymes were added to the water-agar medium to estimate their influences on Selected fungi as described by Mahmoud et al. (2004). About 1 ml of pure enzymes from bacteria were mixed with 0.1 of the spore suspension (4x10⁵ spore/ml). The mixture was spread on the surface of water agar using glass spreader spore germination. Percent of *Trichophyton mentagrophytes, Microsporum gallina, Candida albicans, Fusarium oxysporum, Trichoderma harzianum* and *Aspergillus fumigates* was calculated, after 2, 4, 6, 8 and 16 h, by utilizing the light microscope (WZ 25 IC 280, Germany).

**Statistical analysis:**

Data were represented by the mean ± standard deviation of three independent experiments. Statistical analysis and the difference between mean values were evaluated utilizing the Student’s t-test. Differences were considered significant at $p \leq 0.05$.

**Results**

Most chitinase manufacturing bacteria were collected from various sources on the Mineral chitin agar medium. The most active isolates for chitin degradation on agar medium were isolates AMM1, AMM2, AMM8, AMM31 and AMM37 (Figure 1). After screening in the broth medium for 5 days, the isolate AMM1 showed the highest chitinase activity (2.230 u/ml) and the lowest activity was recorded by the isolate AMM37.

The isolate AMM1 was characterized and identified as *Alcaligenes aquatilis*. It was a Gram-negative, aerobic, rod-shaped motile bacterium with one or more peritrichous flagella. The temperature range was 20-37°C and the optimum was 30°C while G+C content was 56%. The chosen bacterial isolate AMM1 is linked to the genus *Alcaligenes*. By using 16S rRNA, it identified as *Alcaligenes aquatilis* AMM1 with a similarity level 95% to *Alcaligenes aquatilis* (gi: 559795384) (Figure 2).

**Purification of the enzyme:**
The selected isolate AMM1 was cultivated in Mineral chitin broth medium B (pH 7 for 5 days in shaking incubator at 120 rpm) and kept at 37°C for bacterium isolate. The Chitinase was precipitated by ammonium sulfate (80%), then centrifuged. The precipitate was gathered using a cellophane bag, washed in a baker containing distilled water and placed in the refrigerator. The water is changed daily until the enzyme is becoming pure by Sephadex G100 column chromatography. The active fractions with Chitinase activities were gathered. The protein was estimated. The active fractions of Chitinase activities with high absorption at 280 nm were 40-53, thus they were gathered, freeze dry and kept at 4°C till utilized. The dried fractions that revealed the highest activity were utilized for enzyme properties and molecular weight estimation.

The molecular weight of Chitinase:
The pure enzyme was gathered, freeze dry and analyzed. The molecular weight was 46 for the enzyme obtained from AMM1 (Figure 3).

Enzyme properties:
After obtaining the purified enzyme, the properties of the enzyme were studied. The effect of temperature, pH, enzyme and substrate concentration and concentration of heavy metals on the enzyme activity were studied.

As the temperature increased (from 30 to 40°C) the enzyme activity achieved by the bacterial isolate increased. The obtained results presented implied that the ideal temperature for Chitinase from bacterial isolate AMM1 was 40°C (relative activity 110%). The lowest activities were obtained at 4°C and 10°C. The ideal pH presented for the Chitinase enzyme of the bacterial isolate AMM1 was pH 7 (100%). The lowest activities were obtained at pH 4 and pH 10.

The influence of various enzyme concentrations on the activity of Chitinase was studied. The activity was progressively elevated by rising the enzyme concentration from 0.1 up to 0.5 ml. Statistical analysis showed that the enzyme concentration affected significantly enzyme activity and maximum activity was at a concentration of 0.5 ml (relative activity 172%). The lowest activities were obtained at a concentration of 0.1 ml and 0.2 ml. The results of the effect of different enzyme influence of various substrate concentrations on Chitinase activity was studied. The activity was increased progressively elevated by rising the enzyme concentration ranging from 0.3 up to 0.5 ml. Statistical analysis showed that the results of the effect of different substrate concentration was the enzyme concentration affected significantly enzyme activity and the maximum activity was at 0.5 ml of the substrate.

To study the influence of heavy metal ions on enzyme activity, the reaction mixture contained enzyme solution (0.3 ml) and soluble chitin (0.5 ml) at pH 7 (the ideal pH value was estimated from the former experiment). Effect of heavy metal ions (e.g., Cu++, Zn++, Co++, Ca++, Fe++, Hg++ and EDTA++) (1mM) were evaluated by mixing them to the reaction medium. The activity of the has metal was measured as described previously. The obtained results presented which indicated that heavy metal ions exhibited
different effects on the enzyme activity. Fe$^{++}$ enhanced the enzyme activity almost exerted no obvious effects from bacteria isolated AMM1 (relative activity 931%). The lowest activities were obtained from heavy metal ions on Cu$^{++}$ and Ca$^{++}$.

**Biocontrol of some fungal pathogens using the select bacterial AMM1:**

Studying the biocontrol process of some fungal pathogen using the selected bacterial isolate AMM1.

The perpendicular method was used Antagonistic activity between bacteria AMM1 and some fungal pathogens (Figure 4).

**Effect of the purified enzyme from the selected bacteria isolates on % of spore germination:**

Effect of Chitinase on the % of spore germination of *T. mentagrophytes, M. gallina, C. albicans, F. oxysporum, T. harzianum* and *A. fumigatus*. The % of germination was calculated, after 2, 4, 6, 8 and 16 h. after examining using the light microscope (Table 1). The presence of Chitinase decreased the % of spore germination.

Table 1. Effect of a purified enzyme from the selected bacteria AMM1 on pathogenic fungi spores on the agar water media, AMM1 isolation pathogenic fungi spore germinations on the water agar medium.

| Tested fungi                      | Spore germination (%) |
|-----------------------------------|-----------------------|
|                                   | 2 hr  | 4 hr  | 6 hr  | 8 hr  | 10 hr | 12 hr |
| **Candida albicans**              |       |       |       |       |       |       |
| Control                           | 39.222| 49.985| 62.464| 77.864| 89.356| 99.099|
| Treated                           | 36.263*| 43.406*| 58.241*| 67.032*| 67.032*| 67.032*|
| **Trichophyton mentagrophytes**   |       |       |       |       |       |       |
| Control                           | 33.000| 48.000| 58.860| 69.000| 72.000| 88.860|
| Treated                           | 24.500*| 44.000| 52.500*| 67.000| 67.000*| 67.000*|
| **Microsporum gallina**           |       |       |       |       |       |       |
| Control                           | 39.000| 56.860| 61.760| 88.000| 89.976| 95.000|
| Treated                           | 38.974| 50.256*| 60.512| 82.051| 82.051*| 82.051*|
| **Fusarium oxysporum**            |       |       |       |       |       |       |
| Control                           | 22.000| 32.000| 39.970| 66.000| 76.960| 89.850|
| Treated                           | 18.090*| 24.120*| 29.648*| 60.301*| 60.301*| 60.301*|
| Trichoderma harzianum             |       |       |       |       |       |       |
| Control                           | 28.000| 33.096| 66.86 | 77.000| 84.000| 99.120|
| Treated                           | 23.611*| 30.555| 60.301| 65.416*| 65.416| 65.416*|
| Aspergillus fumigatus             |       |       |       |       |       |       |
| Control                           | 32.000| 47.000| 58.000| 68.000| 79.000| 88.876|
| Treated                           | 26.400*| 34.000*| 42.400*| 44.000*| 60.060*| 60.200*|

*: significant results at p<0.05:

**Discussion**
Chitinase from bacteria can prohibit the growing rate of numerous fungal diseases that cause a dangerous threat to crop generation and human health worldwide. Natural bio fungicides can supersede manufactured fungicides and can be utilized to complement the currently utilized fungicides. Out of 40 bacterial isolates, *Alcaligenes aquatilis* was isolated from shrimp shells collected from marine water and identified as described by Williams *et al.*, (1984). Identification was confirmed using 16S rRNA which is an excellent method for bacterial identifications, (Bosshard *et al.*, 2006). As it is well known, insects are a rich resource of chitinase manufacturing microorganisms, especially bacteria (Konagaya *et al.*, 2006) and fungi (Pinto *et al.*, 1997). In the current investigation, forty bacterial isolates were obtained from different resources on the chitin agar medium. Also, Aly *et al.*, (2011) obtained many chitinase producing bacteria on the same medium.

Among the different media tested Mineral chitin broth media (A, B, C, D, E and F) for *Alcaligenes aquatilis & Kitasatospori* sp. Mineral chitin broth media B was found to be the best media for growth and Chitinase production. It has been suggested that for most microorganisms the optimum chitin concentration for Chitinase induction is in the range of 1-2% (Sandhya, *et al.*, 2005). Out of the different colloidal chitin concentrations ranging from 0.1 to 10% (w/v), maximum enzyme production was at a concentration of 1%. Similarly, when chitin concentrations were varied from 1.25 to 15 g/l, it was observed that isolate AXX produced maximum Chitinase with 10-15 g/l chitin (Vaidya *et al.*, 2001). The type and nature of carbon source is one of the most important factors for any type of fermentation process (Pandey *et al.*, 1999). The carbon source represents the energetic source that will be available for the growth of the microorganism.

The molecular weights of microbial Chitinases range from 20,000 to 120,000 with little consistency. The molecular weights of bacterial Chitinases are mostly around 60,000 to 110,000, while those of actinomycetes are mostly 30,000 or lower, fungi are higher than 30,000. The molecular weight of plant Chitinases are mostly around 30,000 (Annamalai *et al.*, 2010). SDS-PAGE analysis of the purified enzyme revealed one protein band with an estimated molecular weight of 42 kDa. Earlier, the molecular weight of other Chitinases from *Bacillus circulans, S. marcescens* and *Micrococcus* sp. AG84 were 45, 57 and 33 kDa respectively (Nawani *et al.*, 2002; Wiwat *et al.*, 1999; Annamalai *et al.*, 2010). In this study, molecular weight of the enzyme was estimated to be almost 46 kDa and 47 kDa for the enzyme obtained from AMM1 and AMB8, respectively. Mandana *et al.*, 2011, found an enzyme solution partially purified by ammonium sulfate 65 % with maximum activity was examined for purity and molecular weight determination by SDS-PAGE. Molecular weight of the enzyme was estimated to be almost 54 kDa. Molecular weights of Chitinases obtained from different strains of *Serratia* were 47–60 kDa (Wang *et al.*, 2002). The molecular weight of Chitinases from *Serratia marcescens* QM13 1466 (Roberts and Cabib, 1982) and *Serratia plymuthica* HRO_C 48 (Frankowski *et al.*, 2001) were 58 kDa and 60.5 kDa respectively. The molecular weight of Chitinases obtained from *Enterobacter* sp. was about 60 kDa. Molecular weight of Chitinase from *Enterobacter* sp. NRG4 (Dahiya *et al.*, 2005), *Enterobacter* sp. G-1 (Park *et al.*, 1997), *Enterobacter agglomerans* (Chernin *et al.*, 1997) and *Enterobacter aerogenes* (Vogelsang and Barz, 1993) were 60 kDa, 60 kDa, 61 kDa and 42.5 kDa respectively.
The role of Chitinase in the biological control of various fungal pathogens has already been established (Yan et al., 2008). Since the Chitinase producing B. pumilus was originally isolated from the rhizosphere, this bacterium could be an ideal candidate for biological control of plant pathogens.

Chitinases can be used as antifungal agents and have environmental and agricultural applications. Kishore et al., (2005) reported that the lytic activity of Chitinase on the cell walls of pathogenic fungi can be correlated with the degree of biological control of these pathogens in vivo. Biopreparations offer an alternative, environmentally friendly strategy for controlling phytopathogens. However, due to several challenges, their application is still limited. Although highly beneficial, the use of microorganisms and their metabolites also has some disadvantages. First, compared to chemical products, biological fungicides require more time to work. Moreover, due to their high selectivity, the costs associated with their use are relatively high (Żydlik, 2008). The environmental conditions, which determine the growth of microorganisms, must be considered. However, different bio fungicides can be combined or implemented using agricultural, physical, and chemical methods to produce a synergistic effect in many ways (Kordowska-Wiater, 2011).

Dahiya et al., (2005) believe that chitinolytic enzymes can be used as supplements for chemical fungicides to increase their effectiveness against pathogenic molds and reduce the required concentrations of these harmful chemicals. In agriculture, the reluctance to use fungicides based on Chitinase is associated with the fear that their impact will be reduced in the natural environment.

Microorganisms producing these enzymes can inhibit the growth of many fungal diseases that pose a serious threat to global crop production. Currently, efforts are being made to discover producers of chitinolytic enzymes. The potential exists that natural bio fungicides will replace chemical fungicides or will be used to supplement currently used fungicides, which would reduce the negative impact of chemicals on the environment and support the sustainable development of agriculture and forestry.

Chitinases may be used to convert chitin-containing biomass into useful (depolymerized) components. Chitinases can be exploited for their use in control of fungal and insect pathogens of plants (Melchers and Stuiver, 2000). It has been reported that there is a strong association between Chitinase activity and fungal population in the soil. Therefore, it appears that Chitinases activity acts as a suitable indicator of the actively growing fungi in the soil (Matroudi et al., 2008).

In the present study, the effect of the purified Chitinase on some pathogenic fungi was studied by more method. The tested pathogenic fungi included Trichophyton mentagrophytes, Microsporum gallina, Candida albicans, Fusarium oxysporum, Trichoderma harzianum and Aspergillus fumigates. The effect of the crude enzyme on the hyphae growth of the tested fungi and the cell morphology were studied, using light microscope. It is clear that, addition of the pure enzyme to fungal growth medium hydrolyzes the cell wall and hyphae become more distorted. The fungus were affected by different degree which may due to the quantity of chitin present in the cell wall and the surface structure of the cell wall. In this connection, Wang et al. (1999) studied the effect of purified Chitinase from Pseudomonas aeruginosa K-187 on hyphal growth of F. oxysporum and A. fumigatus. It was obvious that Chitinase caused extensive
hyphal swelling of both organisms; however, no lysis was observed. On the other hand, the mycelia grew normally in the absence of Chitinase (control). Similarly, Vaidya et al. (2001) found that the Chitinase from *Alcaligenes xylosoxydans* inhibited the growth of *Rhizoctonia bataticola*. In 2002, Wang et al., (2002b) studied the effect of the crude chitinase from *B. subtilis* W113 on the hyphal growth of *F. oxysporum*. It was observed that the hyphae of *F. oxysporum* growing in the absence of the crude Chitinase did not show any obvious growth aberrations. However, an abnormal hyphal swelling was observed when the crude Chitinase was added to the culture broth of *F. oxysporum*. The abnormal hyphae distinguished itself from that of the control. In some instances, extensive degradation of *F. oxysporum* hyphae, or lysis of the hyphal tips caused by the presence of the crude Chitinase were also observed. Dahiya et al. (2005b) found that the Chitinase produced by *Enterobacter* sp. NRG4 was highly active toward *F. moniliforme*, *A. niger*, *Mucor rouxi*, and *Rhizopus niger*.

The Chitinase produced by four bacterial isolates, identified as *B. licheniformis*, *Stenotrophomonas maltophilia*, *B. licheniformis* and *B. thuringiensis* suppressed the growth of the pathogenic fungi *Rhizoctonia solani*, *Macrophomina phaseolina*, *F. culmorum*, *Pythium* sp., *Alternaria alternata* and *Sclerotium rolfsii* (Kamil et al., 2007). In green-house experiment, *B. licheniformis* significantly reduced the damping off disease caused by *Rhizoctonia solani* (Kamil et al., 2007). Similarly, Chang et al. (2010) studied the antifungal activity and enhancement of plant growth by *B. cereus* QQ308 grown on shellfish chitin wastes. They found that *B. cereus* QQ308, which secreted a complex of hydrolytic enzymes, including chitinase, chitosanase and protease, inhibited the growth of several important soil-borne fungal plant pathogens including *F. oxysporum*, *F. solani*, and *Pythium ultimum*. The *B. cereus* QQ308 or its culture supernatant has potential as a fungicide active against numerous plant pathogenic fungi and is expected to be useful in biological control. However studies have shown that the combination of two chitinolytic bacteria *Paenibacillus* sp. 300 and *Streptomyces* sp. 385 is more effective against *F. oxysporum* causing cucumber wilt than individual strains or other combinations (Singh et al., 1999). According to El-Tarabily et al., (2000) the combination of *S. marcescens*, *Streptomyces viridodiasticus*, and *Micromonospora carbonacea* strains effectively inhibited the growth of *Sclerotinia minor* responsible for vegetable rot. Kishore et al., (2005) described a synergistic effect of *Bacillus circulans* GRS 243 and *S. marcescens* GPS 5, the combination which inhibited the growth of the mold fungus *Phaeoisariopsis personata* when used as a prophylactic on the leaves. Forest protection relies on bio fungicides containing *Trichoderma* species, whose spores effectively inhibited the growth of fungi causing canker when mixed with beech sawdust and applied to the soil (Kapuściński, 2002).

In conclusion, Chitinases has an extensive range of applications currently in bio control, medical field, and degradation of pollutant, single cell protein production, bio pesticides and protoplast isolation. The Chitinase activity in bioconversion of shellfish waste to NAG was carried out.

### Declarations

#### Ethical Approval
Conflict of interest, the author declares that she has no conflict of interest.

**Consent to Participate**

Not applicable

**Consent to Publish**

All authors agree for publish

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing Interests**

There is no Competing Interests

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**Authors Contributions**

Magda M. Aly conceived of the presented idea, developed the theory and performed the computations. Also discussed the results and contributed to the final manuscript

Amna A. Seddiq conceived of the presented idea and discussed the results and contributed to the final manuscript

Afra M. Baghdadi verified the analytical methods and investigate [a specific aspect] and supervised the findings of this work.

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**Figures**

![Figure 1](image_url)

**Figure 1**

Decomposition of chitin on Mineral chitin agar medium by five different bacterial isolates.
Figure 2

The selected bacterium AMM1 (A) beneath the Scanning Electron Microscope and (B) Phylogenic tree of the tested bacterium, the isolate AMM1.
Figure 3

A: Purification of the Chitinase using column chromatography. B: SDS-PAGE analysis of Chitinase obtained from bacteria, Lane 1: partial purified Chitinase by ammonium sulfate 80% of cultrate of AMM1, Lane 2: Chitinase of isolating AMM1 and lane 4: Markers.
Figure 4

Biocontrol of some fungal pathogens using the selected bacterial AMM1.