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Chitinase production by endophytic *Streptomyces aureofaciens* CMUAc130 and its antagonism against phytopathogenic fungi

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Abstract - More than three hundred isolates of endophytic actinomycetes were screened for their potential for chitinase production. The strain identified as *Streptomyces aureofaciens* CMUAc130 was the most effective amongst those investigated. This isolate was selected for a more detailed study of chitinase production and its effectiveness in fungal cell wall lysis. Production of the chitinase was optimal with 1% colloidal chitin concentration, at 30-40 ºC, pH 6.5-7.0 and 100-150 rev min^{-1} shaking. *N*-acetylglucosamine was a good inducer and expression of the enzyme complex was repressed by several mono- and disaccharides including lactose, mannose, glucose, cellobiose, arabinose, raffinose, sucrose, xylose and fructose. Addition of pectin, starch and carboxymethyl cellulose to the colloidal chitin-containing medium, increased chitinase production. The enzyme tolerated a wide range of temperature (30-50 ºC) and pH (5.5-8). Among various divalent cations Hg^{2+} Cd^{2+} and Ni^{2+} completely inhibited the purified enzyme while Mg^{2+} stimulated its activity. The crude or purified enzyme had potential for cell wall lysis of many phytopathogenic fungi tested.

Key words: antifungal activity, chitinase, *Streptomyces aureofaciens.*

INTRODUCTION

Chitin is a polymer of unbranched chains of β-1,4-linked 2-acetamido-2-deoxy-D-glucose (GlcNAc; *N*-acetylglucosamine; NAG) and is a major structure component of most fungal cell walls. It is hydrolysed by two separate enzymes catagorized as chitinases: an endo-chitinase (EC 3.2.1.14) which produces soluble low molecular weight multimers of NAG, the dimer *N,N*-diacetylchitobiose being dominant and chitobiase (EC 3.2.1.30), which hydrolyses the intermediate to NAG. These chitinolytic enzymes have been found in microorganisms and plants (Selitrennikoff, 2001). Some of the most potent chitinase producers are *Serratia marcescens*, *Streptomyces* sp. and *Trichoderma harzianum*. Microbial chitinases are also commercially available (Flach *et al*., 1992; Cohen, 1993). During the last decade, chitinases have received increased attention because of their wide range of applications.

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As the fungal cell wall is rich in chitin (Peberdy, 1990), it could be a potential target in biocontrol of fungal phytopathogens. The enzymes could be used directly in biological control of microorganisms (Ordentlich *et al*., 1988; Gomes *et al*., 2000) or indirectly using purified protein (Ueno *et al*., 1990; Gunaratna *et al*., 1994; Gomes *et al*., 2001) or through gene manipulation (Oppenheim and Chet, 1992; Lorito *et al*., 1994; Tsujibo *et al*., 2000; Viterbo *et al*., 2001).

Endophytic actinomycetes have been isolated from various plant tissues and some of them have potential as biocontrol agents against phytopathogenic fungi (Sardi *et al*., 1992; Shimizu *et al*., 2000). Some strains of endophytic actinomycetes that produced chitinase might protect against phytopathogenic fungi in plant tissue by this property. Thus the role of chitinase of endophytic actinomycetes in antifungal activity should be studied as they may endow their host plant with some natural protection against infections.

MATERIALS AND METHODS

Screening of chitinase producing actinomycetes. Three hundred and seven endophytic actinomycetes were isolated from leaves, stems and roots from healthy plants (Sardi *et al*., 1992; Shimizu *et al*., 2000), and were maintained on ISP-2 agar slants (Shirling and Gottlieb, 1966). Screening for chitinase production of all the isolates was by plate agar assay and followed by tests in broth for chitinase production. The colloidal chitin medium contained (g l^{-1}): colloidal chitin 15; yeast extract 0.5; $(NH_4)_2SO_4$ 1; $MgSO_4 \cdot 6H_2O$ 0.3; KH_2PO_4 1.36; and agar 15, as required. Practical grade crab shell chitin powder (Sigma) was used to prepare colloidal chitin (Berger and Reynolds, 1958) as a substrate for growth and enzyme assay. Enzyme production was carried out in shake culture (50 ml medium/250 ml Erlenmeyer flask) incubated at 30 ^oC in the incubator shaker (Sanyo Gallenkamp PLC, England) at 150 rev min^{-1} for 7 d. Spores were inoculated to a concentration of 10^5 ml^{-1}. Physiological conditions including temperature, shaking speed, substrate concentration and carbon source were studied to obtain maximum enzyme production from the test chitinase-producing strain. Each test was performed in triplicate. The results were presented as mean values of triplicate tests + SD.

Enzyme assay. Colloidal chitin was used as a substrate to assay chitinase activity: 0.1 g in 1 ml of phosphate buffer (pH 7.0) was incubated with 0.5 ml of enzyme at 45 ^oC for 60 min. The reducing sugars in the reaction mixture were measured by Somogyi Nelson's colorimetric method (Hodge and Hofreiter, 1962). One unit of enzyme activity was defined as the amount of enzyme that catalysed the release of 1 μmol of N-acetylglucosamine ml^{-1} in 60 min.

Enzyme purification and kinetics. *S. aureofaciens* CMUAc130 appeared to be a high level chitinase producing strain, so it was further studied. Spores ($5x10^7$) of *S. aureofaciens* CMUAc130 were inoculated into 500 ml of colloidal chitin medium and incubated at 30 ^oC in the incubator shaker at 150 rev min^{-1} for 7 d. The culture medium was then filtered through Whatman No.1 filter paper and the culture filtrate was centrifuged in a refrigerated centrifuge at 4 ^oC for 20 min at 5000 rev min^{-1}

to remove any remaining cell debris. The culture filtrate obtained was used as the crude enzyme.

The crude enzyme sample was precipitated by 80% ammonium sulphate and purified by Sephadex G-75 column chromatography. The fractions with chitinase activity were pooled and lyophilized by Dura-dry freeze-dryer (FTS systems, USA) for further biochemical characterization by incubation at different temperatures and pH. The purity of the chitinase protein was determined by SDS-PAGE by using 5% stacking and 15% separating gels (Laemmli, 1970), and the gels stained with Coomassie Brilliant Blue R-250 in methanol/acetic acid/water (40:10:50, by vol.).The effect of various divalent cations (Mn^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+} and Hg^{2+}) and other inhibitors such as 2-mercaptoethanol and EDTA on enzyme activity was investigated. Purified enzyme was incubated at 37 ºC with 5 mM of each divalent cations for 10 min and then the enzyme activity was assayed. Chitinase activity on several chitin-derived substrates and the chito-oligosaccharide was determined. The substrates used for this study were 1% (w/v) of ball milled chitin, *Schizosaccharomyces* sp. cell walls, crude chitin (Sigma), colloidal chitin, CM-cellulose and 0.25 mM of chito-oligosaccharide; *N*,*N*-diacetylchitobiose, *N*,*N*,*N*-triacetylchitotriose and *N*,*N*,*N*,*N*-tetraacetylchitotetraose. Each substrate was prepared in 0.05 M sodium phosphate buffer, pH 7.0. A single reaction contained 0.5 ml of purified enzyme plus 0.5 ml of the test substrate and incubated at 37 ºC for 1 h. The amount of *N*-acetylglucosamine released was determined using the procedure described.

The chitinase enzyme obtained from *S. aureofaciens* CMUAc130 was tested for its potential to act as a biocontrol agent against important fungal phytopathogens by virtue of its lytic action on the chitin component of the cell walls.

Preparation of fungal cell walls. The fungi *Colletotrichum musae*, *Fusarium oxysporum*, *Bipolaris* sp., *Drechslera* sp., *Rhizoctonia* sp., *Sclerotium* sp. and *Candida albicans* ATCC 90028 were grown in potato dextrose broth, incubated at 30 ºC, and then harvested after 72 h growth. Cell walls were obtained from the mycelia of the above species as described by Skujins *et al.* (1965). For *C. albicans*, the culture medium was centrifuged in a refrigerated centrifuge at 5000 rev min^{-1} for 20 min at 4 ºC. The cells were washed by distilled water for three times. The mycelium of each fungus and the cells of *C. albicans* were autoclaved and subjected to ultrasonic disintegration in an ice bath for 6 min in 15 cycles each of 30 s in ultrasonicator (Karl kolb, Germany). The pellet obtained by centrifugation at 5000 rev min^{-1} for 30 min at 4 ºC was washed three times with distilled water and dried.

Colloidal chitin along with chitin present in the fungal cell wall in a 1:3, 1:0 and 0:3 ratio was used for enzyme production in the medium. Flasks were inoculated with *S. aureofaciens* CMUAc130 and incubated at 30 ºC in an incubator shaker at 150 rev min^{-1} for 7 d. Culture filtrates were tested for enzyme activity as described above.

Dissolution of fungal cell wall. The ability of the culture filtrate to lyse different fungal cell walls and thereby release *N*-acetylglucosamine was tested in a reaction mixture containing 20 mg of fungal cell wall and 50 ml of culture filtrate in a 250-ml flask. The flask containing the reaction mixture was incubated for 24 h at 37 ºC.

N-acetylglucosamine was measured as according to the method of Reissig *et al*. (1955). Heat-inactivated culture filtrates containing fungal cell walls were used as the control.

Inhibition of fungal growth by purified enzyme extract. *Fusarium oxysporum*; the causative agent of wilt of wheat was cultured on potato dextrose agar (PDA). Mycelial discs of 6 mm diameter of this fungal pathogen was transferred from PDA onto the center of PDA plates which was punched of 6 mm diameter of the agar medium 3 cm away from mycelium disks. In the case of colloidal chitin basal medium was punched as the same position of PDA plates but not transfer the mycelium disks onto the center of plates. Fifty microliters of the purified chitinase of *S. aureofaciens* CMUAc130 was placed in the wells at the concentration 5, 2.5 and 1.25 mg ml^{-1}. The plates were incubated at 30 ^{o}C in moist chamber for 5 d; controls were 5 min-boiled enzyme extract. Inhibition of mycelium growth was observed over 5 d of incubation.

Microscopy. *Fusarium oxysporum* was cultured on PDA. A mycelial disk of 6-mm diameter was transferred onto a glass slide and incubated at 30 ^{o}C in a moist chamber. After 48 h growth, a sterilized solution of the purified chitinase (1.5 U) of *S. aureofaciens* CMUAc130 was overlaid and incubated at 37 ^{o}C for 12 h in moist chamber. Morphological modification of the mycelial structures of the fungus were observed under a light microscope (400X) and compared with a 5 min-boiled chitinase control.

RESULTS

Screening for extracellular chitinase production of endophytic actinomycetes
Three hundred and seven isolates of endophytic actinomycetes were grown on a basal medium containing colloidal chitin. After 7 d the chitinolytic zones around the actinomycete colonies were observed and measured (data not shown). Fourteen isolates produced a chitinolytic zone more >5 mm diameter. These isolates were selected for further study of the level of chitinase production in colloidal chitin broth. Cultures were incubated at 30 ^{o}C and shaken at 150 rev min^{-1}. After 7 d, the cultures were harvested and chitinase present in the culture filtrates were determined. All fourteen isolates produced chitinase (Table 1). The best strain for chitinase production was strain CMUAc130, which produced nearly 2-6 times more enzyme activity than any other isolates. All subsequent experiments involved the use of this strain.

Morphology, chemotaxonomy, sequence comparisons and phylogenetic analysis
Based on the results of a morphological observation, detection of LL-type diaminopimelic acid in the whole-cell extracts and 16S rDNA analysis, it was concluded that strain CMUAc130 is phylogenetically closely related to *S. aureofaciens* (the sequence similarity levels were 97%) (Taechowisan *et al*., 2003; Taechowisan and Lumyong, 2003).

TABLE 1 – Screening endophytic actinomycete isolates for chitinase production in shake culture. The strains selected based on the size of chitinolytic zones produced by samples of colloidal chitin broth from cultures incubated at 30 °C for 7 days. The results are means of three replicates ± SD

Endophytic actinomycete isolates	Enzyme activity (mU ml^{-1})
CMUAc036	0.0176 ± 0.0035
CMUAc042	0.0233 ± 0.0041
CMUAc075	0.0320 ± 0.0045
CMUAc130	0.0833 ± 0.0045
CMUAc169	0.0266 ± 0.0025
CMUAc174	0.0416 ± 0.0025
CMUAc196	0.0420 ± 0.0020
CMUAc197	0.0250 ± 0.0026
CMUAc230	0.0386 ± 0.0040
CMUAc237	0.0450 ± 0.0040
CMUAc249	0.0226 ± 0.0045
CMUAc259	0.0420 ± 0.0036
CMUAc292	0.0346 ± 0.0032
CMUAc294	0.0393 ± 0.0025

Physiological optimization of chitinase production

Maximum levels of *S. aureofaciens* CMUAc130 chitinase were achieved by the addition of 1% colloidal chitin, at 30-40 ^{o}C with 100-150 rev min^{-1} shaking in pH 6.5-7.0 culture after 7 d incubation (data not shown). The effect of various sugar additions along with 1% colloidal chitin on chitinase production was tested. There was a decrease in chitinase production with all sugars tested, except *N*-acetylglucosamine. *N*-acetylglucosamine plus colloidal chitin increased chitinase production but the amino sugar alone failed to induce the enzyme production (Fig. 1). Several monosaccharides and disaccharides including glucose, mannose, cellobiose, arabinose, raffinose, xylose, lactose, fructose and sucrose showed partial suppression of chitinase production (Fig. 2) but 0.3% addition of pectin, starch and carboxymethyl cellulose to the colloidal chitin-containing medium, increased chitinase production (data not shown) relative to glucose. Among the different chitin substrates, colloidal chitin is the best substrate for chitinase production (Fig. 3).

Purification and characterization of chitinase

The enzyme was concentrated by precipitation with 80% $(NH_4)_2SO_4$ and was further purified on Sephadex G-75 to yield three fractions; of these one contained all the chitinase activity (Fig. 4). The purified enzyme was active over a wide range of temperature (30-50 ^{o}C) and pH activity (5.5-8) with optima at 37 ^{o}C and pH 7 (data not shown). No significant difference in inhibition between the crude and purified enzyme was found among various divalent cations except Cd^{2+}, Ni^{2+} and Hg^{2+},

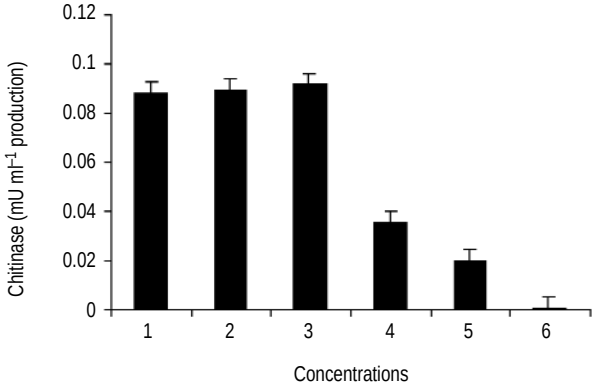

FIG. 1 – Effect of different concentrations of NAG and colloidal chitin on chitinase production after incubation at 30 ºC for 7 days. 1: 0%; 2: 0.1%; 3: 0.5%; 4: 1%; 5: 1.5% of NAG and 1% colloidal chitin. 6: 0.5% of NAG without colloidal chitin. Each value is the mean of triplicate tests with SD error bars indicated.

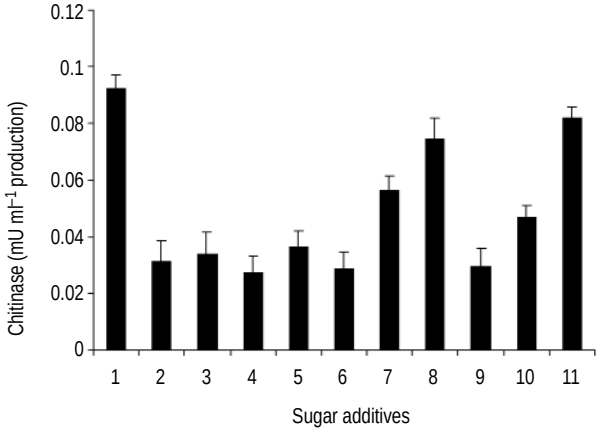

FIG. 2 – Effect of 0.5% sugar additives and 1% colloidal chitin on chitinase production after incubation at 30 ºC for 7 days. 1: NAG; 2: arabinose; 3: cellobiose; 4: fructose; 5: glucose; 6: lactose; 7: mannose; 8: raffinose; 9: sucrose; 10: xylose; and 11: control, without sugar additive. Each value is the mean of triplicate tests with SD error bars indicated.

while Mg^{2+} enhanced the chitinase activity of both the crude and purified enzyme (data not shown). The purified fraction with chitinase activity exhibited a single band on a 15% SDS-PAGE gel by staining with Coomassie blue. One protein band, with an apparent molecular weight of 40 KDa (data not shown), was found only when chitin was used as carbon source. This protein has the same apparent molecular weight as the purified chitinase from *S. aureofaciens* CMUAc130 obtained by

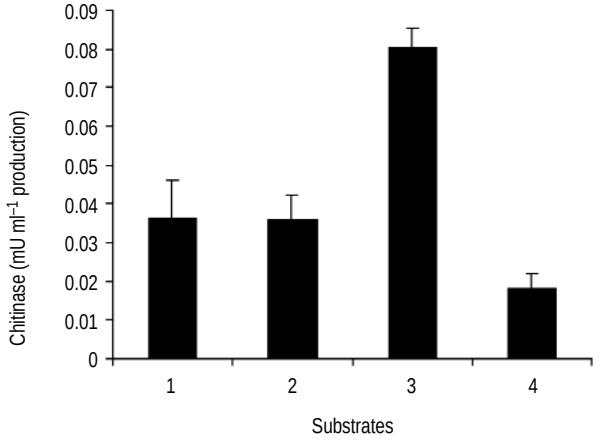

FIG. 3 – Effect of different 1% chitin substrates on chitinase production. 1: ball milled chitin; 2: crude chitin; 3: colloidal chitin; and 4: *Schizosaccharomyces* sp. cell walls. Each value is the mean of triplicate tests with SD error bars indicated.

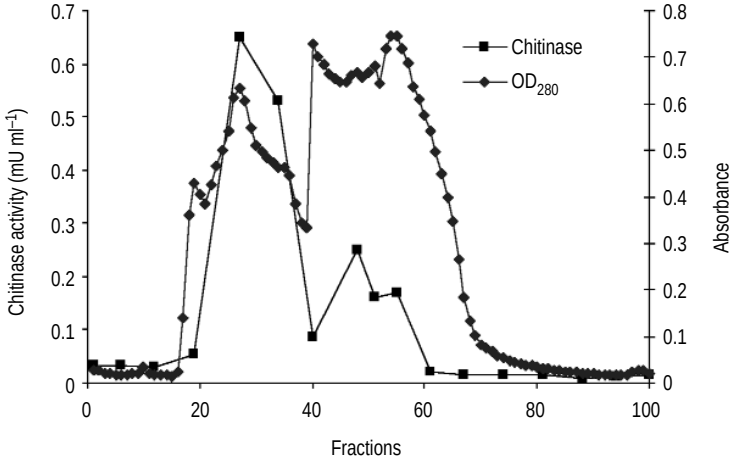

FIG. 4 – Chitinase activity of different protein fractions obtained on Sephadex G-75 gel chromatography.

Sephadex G-75 gel filtration. The chitinase activity on several chitin derived substrates and chitooligosaccharides was investigated and presented in Fig. 5. The substrates most readily hydrolysed were colloidal chitin, ball milled chitin, *N*,*N*,*N*-triacetylchitotriose and *N*,*N*,*N*,*N*-tetraacetylchitotetraose; methylcellulose and chitobiose were not hydrolysed.

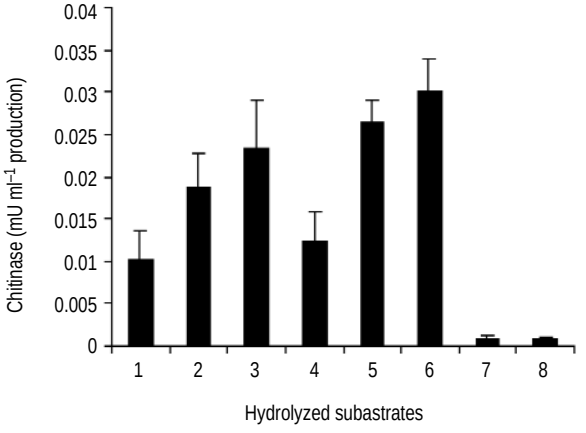

FIG. 5 – Substrate specificity of purified *Streptomyces aureofaciens* CMUAc130 chitinase on several chitin derived substrates and chitooligosaccharides. 1: crude chitin; 2: ball milled chitin; 3: colloidal chitin; 4: *Schizosaccharomyces* sp. cell walls; 5: *N,N,N*-triacetylchitotriose; 6: *N,N,N,N*-tetraacetylchitotetraose; 7: *N,N*-diacetylchitobiose; and 8: CM-cellulose.Each value is the mean of triplicate tests with SD error bars indicated.

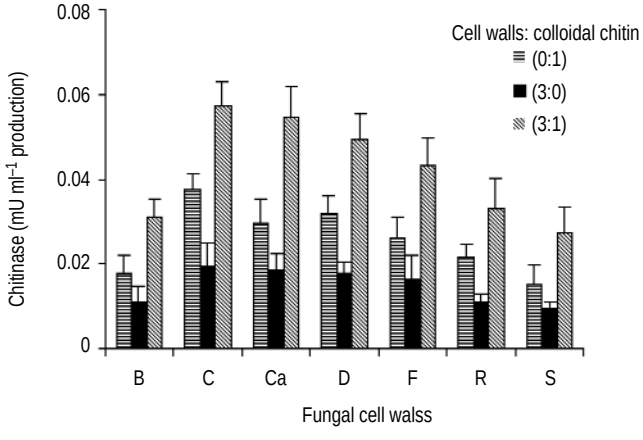

FIG. 6 – Production of *Streptomyces aureofaciens* CMUAc130 chitinase on mixed substrate containing colloidal chitin and chitin derived from different fungal cell walls. B: *Bipolaris* sp.; C: *Colletotrichum musae*; Ca: *Candida albicans*; D: *Drechslera* sp.; F: *Fusarium oxysporum*; R: *Rhizoctonia* sp. and S: *Sclerotium* sp. Each value is the mean of triplicate tests with SD error bars indicated. Ratios shown are expressed as fungal cell walls : colloidal chitin.

Lysis of the fungal cell walls

Improved levels of chitinase production were observed when fungal cell wall chitin was used along with colloidal chitin in a 3:1 ratio (Fig. 6). The culture filtrate thus obtained had chitinase activity. Both colloidal chitin and chitin from fungal cell

TABLE 2 – Activity of *Streptomyces aureofaciens* CMUAc130 chitinase produced in cultures using *Colletotrichum musae* cell walls and colloidal chitin (3:1) as inducer. The activity was measured after incubation the reaction mixture at 37 °C for 24 h. The results are means of three replicates ± SD

Fungal cell wall substrate for dissolution	*N*-acetylglucosamine (μg ml^{-1})
Colletotrichum musae	0.0300 ± 0.0030
Fusarium oxysporum	0.0200 ± 0.0070
Bipolaris sp.	0.0156 ± 0.0072
Drechslera sp.	0.0236 ± 0.0010
Rhizoctonia sp.	0.0066 ± 0.0020
Sclerotium sp.	0.0053 ± 0.0025
Candida albicans ATCC90028	0.0243 ± 0.0010

walls were hydrolyzed. The maximum digestion of fungal cell walls by the crude enzyme extract occurred after a 24 h incubation of reaction mixtures. Crude chitinase preparations from *S. aureofaciens* CMUAc130 cultures grown for 7 d in a medium containing a 3:1 ratio of cell walls to colloidal chitin hydrolyzed chitin prepared from the cell walls of *C. musae* at activity of 0.03 mU ml^{-1} which was the highest chitinase activity among the chitinase produced on fungal cell walls and colloidal chitin (Table 2).

Fungal growth inhibition by the purified chitinase

The purified chitinase from *S. aureofaciens* CMUAc130 were tested for antifungal activity by their ability to inhibit hyphal extension growth of *F. oxysporum*. The inhibition of fungal growth was observed on an agar well diffusion with various concentration of chitinase enzyme (Fig. 7).

Microscopical observation of fungal cell wall lysis

Morphological changes were observed in microslide-culture of *F. oxysporum* treated with the purified chitinase. Inhibition of spore germination and fungal cell wall lysis was noted (Fig. 8A) when compared with the control (Fig. 8B).

DISCUSSION

The ability to degrade chitin is common in soil microorganisms and especially in actinomycetes (Williams and Robison, 1981). This was confirmed in this study as many of the actinomycete isolates proved to be chitinolytic. The strain examined in this work, *S. aureofaciens* CMUAc130, hydrolysed colloidal chitin slowly taking at least 1 week to produce a clear zone in static plate culture. In contrast production of chitinases in liquid cultures was evidently more rapid and prolific. In contrast *Streptomyces* sp. CMUAc196 appeared to produce more enzyme in the agar plate culture, but less in shake cultures. This phenomenon may depend on the different

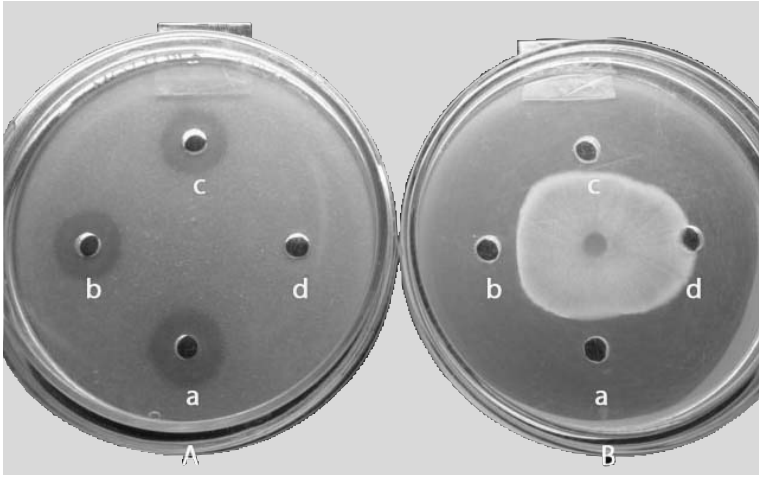

FIG. 7 – Effects of purified chitinase of *Streptomyces aureofaciens* CMUAc130 on chitinolytic activity (A) and inhibition of *Fusarium oxysporum* growth by agar well diffusion method (B) and with a: 5 mg ml^{-1}; b: 2.5 mg ml^{-1}; c: 1.25 mg ml^{-1} of purified chitinase and d: 5 mg ml^{-1} of 5 min-boiled purified chitinase.

forms of chitinase produced by individual strains, because many chitinases have been reported in *Streptomyces* strains (Romaguera *et al*., 1992; Tsujibo *et al*., 2000).

S. aureofaciens CMUAc130 was selected for this investigation because of its very high chitinase productivity and its potential role in antifungal activity (Taechowisan *et al*., 2003; Taechowisan and Lumyong, 2003). Maximum enzyme production at 30-40 ºC is consistent with results reported by other workers (Skujins *et al*. 1965, Gupta *et al*. 1995, Mahadevan and Crawford, 1997; Gomes *et al*., 2001) for chitinases from *Streptomyces* sp. Negligible levels of enzyme were seen upon growth of *S. aureofaciens* CMUAc130 in the absence of chitin. Low constitutive levels probably help induce enzyme production in the presence of chitin (Vasseur *et al*., 1990). Most of the chitinolytic systems reported in the literature are inducible (Ulhoa and Peberdy, 1991; Gupta *et al*., 1995; Mahadevan and Crawford, 1997). Chitinase production by *S. aureofaciens* CMUAc130 was induced by colloidal chitin as well as by low levels of *N*-acetylglucosamine and pectin, starch and carboxymethyl cellulose, confirming the findings of Mahadevan and Crawford (1997). *S. aureofaciens* CMUAc130 has also produced amylase, pectinase and cellulase (data not shown). Among the monosaccharide and disaccharide as additives to colloidal chitin, almost all of them repressed enzyme production. Overall, these data show that *S. aureofaciens* CMUAc130 chitinase is inducible with low constitutive levels and is subject to catabolite repression by sugar. The same phenomena were reported in *Streptomyces* chitinases (Gupta *et al*., 1995; Mahadevan and Crawford, 1997).

The preferred substrate for chitinase production by *Streptomyces* CMUAc130 was colloidal chitin. The enzyme has a higher specificity to this substrate than

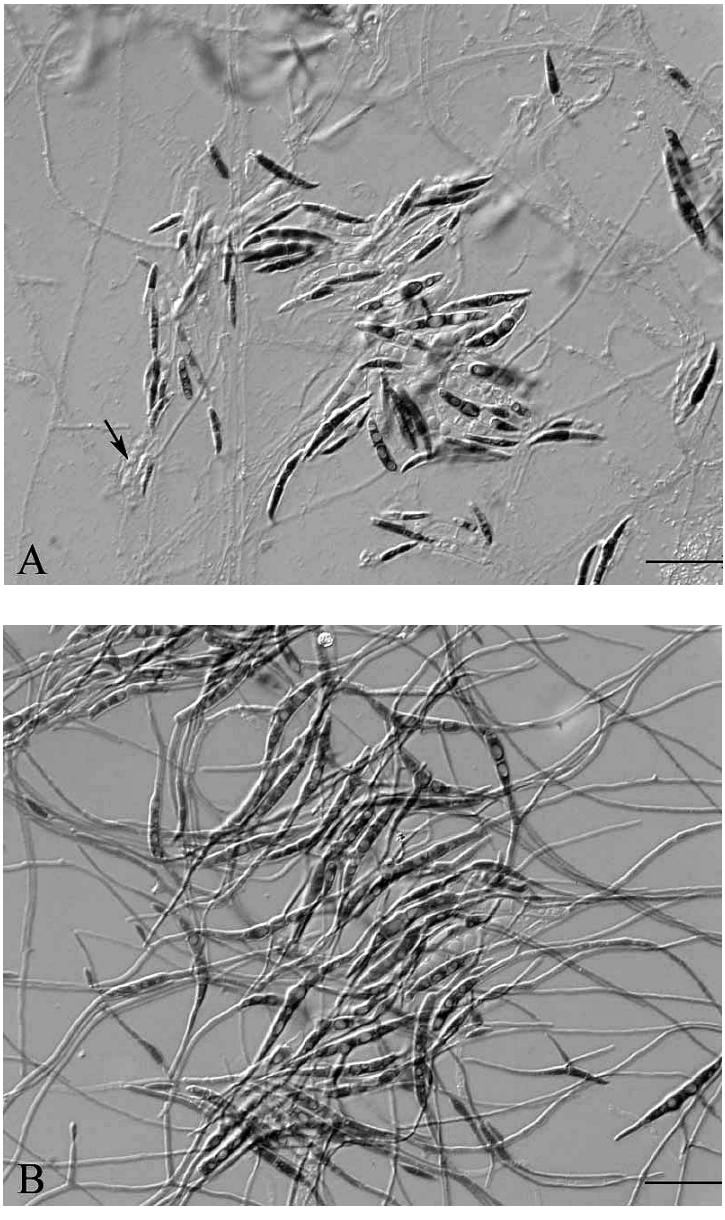

FIG. 8 – Morphological changes of *Fusarium oxysporum* mycelium after treatment for 12 h at 37 ºC with purified chitinase (A) and heat-inactivated purified chitinase (B) of *Streptomyces aureofaciens* CMUAc130. Arrow shown bursting of spore and hyphae. Bar = 10 µm.

other substrate tested e.g. crude chitin from crab shells, ball milled chitin and *Schizosaccharomyces* sp. cell walls. This property confirms the finding that chitinases produced by *Streptomyces viridificans* (Gupta *et al*., 1995), *Streptomyces lydicus* WYEC108 (Mahadevan and Crawford, 1997) and *Acremonium obclavatum* (Gunaratna and Balasubramanian, 1994) also hydrolyse colloidal chitin more rapidly than crude chitin or chitin from fungal cell walls.

The purified enzyme was completely inhibited by 5 mM divalent cations. The increase in activity with mercaptoethanol indicates presence of sulhydryl groups on the active site of the enzyme, confirmed by total inhibition by Hg^{2+}. Similar inhibition and mercaptoethanol enhancement has been reported by Pegg (1982), Ueno *et al*. (1990) and Gupta *et al*. (1995). The activity of the enzyme was also inhibited by the metal ion chelating compound EDTA, suggesting that metals may be important in enzyme stability and/or activity which was difference from the report of Gomes *et al*. (2001).

The substrate specificity of the *S. aureofaciens* CMUAc130 chitinase was investigated. It also capable of hydrolysed several insoluble chitin substrates, with highest activity on ball milled chitin compared to crude chitin and cell wall material from *Schizosaccharomyces* sp. The enzyme was also active on the chitooligosaccharides, chitotriose and chitotetraose, but was inactive towards chitobiose and CM-cellulose. These results indicated that the *S. aureofaciens* CMUAc130 chitinase isolated and purified in this work is specific only for *N*-acetylglucosamine oligomers of more than two units in length. This purified chitinase could be classified as an endochitinase (Oppenheim and Chet, 1992).

Fungal cell walls often contain chitin as a major component, and are susceptible to chitinases (Ueno *et al*., 1990; Gupta *et al*., 1995; Mahadevan and Crawford, 1997; Gomes *et al*., 2001). Antagonistic activity of several *Streptomyces* spp. against a number of fungal pathogenic species has been known for a long time (Crawford *et al*., 1993; Yuan and Crawford, 1995). However, the exact mechanism of this process has not yet been clarified. Whilst the role of chitinase activity against the fungal cell wall is evident (Shapira *et al*., 1989; Lim *et al*., 1991), other carbohydrases e.g. ß-glucanases, or other hydrolytic enzymes such as proteases, could also be involved in the process. In the present study, *S. aureofaciens* CMUAc130 was shown to produce a high level of chitinase when grown in the presence of fungal chitin. The chitinase produced on this substrate was active against all other fungi as measured by the release of sugars from their cell walls (Beyer and Diekmann, 1985; Ordentlich *et al*., 1988; Gupta *et al*., 1995; Mahadavan and Crawford, 1997; Lima *et al*., 1999; Gomes *et al*., 2001; Mansour and Mohamedin, 2001).

The purified chitinase lysed the mycelium and inhibited spore germination of *F. oxysporum.* Reduction in hyphal width was also observed. Different morphological effects on the fungal cell wall have been reported in studies using *Streptomyces* sp. or other microbial chitinases. These effects were mainly spore germination inhibition, bursting of spores and hyphal tips, and germ tube elongation (Lorito *et al*., 1993; Gomes *et al*., 2001). The differences in the effects can be attributed to the different experimental conditions of the tests. This study has shown a probable role for chitinase in the antifungal activity of *S. aureofaciens* CMUAc130. However, antifungal antibiotics produced by *S. aureofaciens* CMUAc130 probably also play a role. The results of the present investigation using the crude and partially purified

chitinase of endophytic *S. aureofaciens* CMUAc130 in controlling fungal growth show that this may be a promising method of biocontrol of some plant pathogens.

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