



## Production of Chitinase Enzyme by some Bio-Control Agents against Five Pathogenic Fungi

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**Abstract**— In the absence of the pathogen or chitin in the growth media, chitinase activities were very limited and mostly in significant, compared with the control. Addition 2% chitin in the growth media, in the absence of the pathogen, resulted in significant increases in chitinase activities. The highest chitinase activity values were obtained by *Trichoderma harzianum* (44.96 unit/ ml), whereas activity values for the other tested Fungal Biocontrol Agents (FBA's) ranged from 4.5 to 19.42 unit/ml. In the presence of the tested pathogens in growth media, chitinase activities significantly increased in all FBA/pathogen treatments, except for *Pythium ultimum*. The highest enzymatic activity was realized by *Trichoderma harzianum*/*Macrophomina phaseolina*, *Fusarium solani* and *Sclerotinia sclerotiorum* treatments (from 56.48 to 59.89 unit/ml). On the other hand, the least chitinase activities were detected by *Gliocladium roseum* with any of the tested seed-borne pathogens (less than 20% of those realized by *Trichoderma harzianum*).

**Keywords:** chitin, chitinase activities, enzymatic activity, FBA/pathogen treatments, seed-borne pathogens

### INTRODUCTION

Fungal pathogens cause serious problems to many economical important crops over the world. Fungicides and chemical substances are widely being used in agriculture and this has led to deteriorating human health, environmental damages, and also may increase the capability of pathogen to resist fungicide [1]. The biological control of fungal plant diseases using microbial antagonists is globally used [2]. It has been observed that various microorganisms such as endophytic *Streptomyces hygroscopicus*, which able to secrete mycolytic enzymes, are considered as biological control agents against plant diseases [1]. Other studies also revealed that many endophytic micro-organisms have been shown to be biocontrol agents of fungal plant pathogens as they able to secrete a wide range of lyticenzymes. The digestive microbial enzymes are fundamentally used in many important industrial applications and food processes [3]. Chitinase is a group of lytic enzymes that produced by many endophytic micro-organisms which known as a source of biocontrol agents [4]. This enzyme degrades chitin directly to low molecular weight [5] and breaks down glycosidic bonds in chitin [1]. It has been reported that there are a broad range of microorganisms able to produce chitinase, these including bacteria such as *Bacillus*, *Serratia*, *Enterobacter*, fungi (*Trichoderma* and *Aspergillus*), and higher plants, insects, crustaceans, invertebrates and some vertebrates [5]. In addition, [1] currently investigated the antifungal activity of purified industrial enzyme chitinase that produced by *Streptomyces* against phytopathogens including *Rhizoctonia solani*, *F. oxysporum*,



*Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *Sclerotinia sclerotiorum*, *Phytophthora parasitica* and *Botrytis cinerea*. Consequently, the chitinase were found to inhibit the growth of all tested phytopathogenic fungi. Furthermore, chitinases play an important role in a variety of biotechnological sectors, particularly in the biocontrol of fungal pathogens [6]. Other research also revealed that chitinase presents in some plant resistance against fungal pathogens and also implicated in pathogenesis of many viruses [3].

## MATERIALS AND METHODS

**The applied fungal biocontrol agents:** The biocontrol fungal agents used in this experiment were *Trichoderma harzianum*, *T. viride*, *T. koningii*, *T. aureoviride*, *T. longibrachiatum* and *Gliocladium roseum*. The fungal isolates were obtained from Mycological Center, Assiut University, Egypt. The pathogenic fungi namely, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium ultimum*, *Macrophomina phaseolina* and *Fusarium solani* were isolated from cucurbit seeds.

### Some biochemical properties of the fungal bio-agents:

**Chitinase enzyme activity:** Chitinase activity was determined according to [7] by dintrosalicylic acid (DNSA). One ml reaction mixture contained 0.5 ml of 0.5 % shrimp shell colloidal chitin in 0.05 M citrate buffer (pH 5.5) and 0.5 ml crude enzyme . Mixture was mixed well and was incubated at 37°C for 30 min. Reaction was arrested by adding 1 ml DNSA reagent, followed by heating at 100°C for 10 min. Control reaction contains 0.5 ml buffered substrate . Crude enzyme (0.5 ml) was added to control reaction after the addition of DNSA. Colored solution was measured at 540 nm. One unit of chitinase activity is defined as the amount of enzyme that is required to release 1  $\mu$ mol of N acetyl-B-D glucosamine per min from 0.5% dry colloidal chitin solution under assay conditions.

## RESULTS

### *Chitinase activities in different FBA-pathogen interactions:*

This investigation was carried out to study chitinase activities of some fungal biocontrol agents (FBA's) in the presence of damping-off pathogens. The tested FBA's were *T. harzianum*, *T. viride*, *T. koningii*, *T. aureoviride*, *T. longibrachiatum* and *G.roseum*. The damping-off pathogens applied throughout this research were *R. solani*, *S. sclerotiorum*, *P. ultimum*, *M. phaseolina* and *F. solani*. The obtained data were presented in **Table (1)**, which can be concluded as follows: Control experiment revealed that, chitinases activity of FBA's in cultural filtrates in the absence of the pathogen and without addition of chitin substrate were almost very limited and insignificant. However, growing any of the tested biocontrol agents in PD media amended with chitin at conc. 2% significantly stimulated the activity of chitinases, compared with the control without chitin amendment. Moreover, enzyme activity in amended control was significantly differed among the tested biocontrol agents. *T. harzianum* realized an exclusive high chitinase activity (44.96 unit / ml), compared with the other tested FBA's only (4.5 to 19.42 unit / ml). In general, growing of FBA's and pathogens in dual cultures significantly stimulated chitinase activities. This was true for all FBA / pathogen combinations, except FBA's / *P. ultimum* which did not stimulate such activities (1.68-5.02 unit/ml). Chitinase activity values attained the highest levels in *T. harzianum* as stimulated by *S. sclerotiorum*, *M. phaseolina* and *F. solani*. This trend was true for each of



the tested FBA's. However, *R. solani* appeared to induce the least chitinase activity compared with all the tested FBA's, compared with these other tested pathogens. The highest chitinase activity values were found to be induced by both *T. harzianum* and *T. viride*, compared with the other tested FBA's. However, differences in chitinase activity values between *T. harzianum* and *T. viride* were insignificant, in all the tested pathogens. Chitinase activities were intermediate in both *T. koningii* and *T. aureoviride*, compared with the other FBA's, whereas *G. roseum* expressed the least values of chitinase activity (about 20% of those of *T. harzianum* and *T. viride*).

### DISCUSSION

The obtained results confirmed the previous findings on the role of chitinases in biocontrol. High chitinase activities were induced by *T. harzianum*, *T. viride* and *T. koningii*, particularly if grown with *S. sclerotiorum*, *M. phaseolina* and *F. solani*. On the other hand, insignificant induction of chitinases was obtained with *P. ultimum*. This might be explained by the fact that *P. ultimum*, an oomycetes lacking significant amounts of chitin in its cell wall. *G. roseum* was proved to be the least effective in chitinase activities, compared with the other tested FBA's. Production of chitinase by *Trichoderma spp.* and the possible role of chitinolytic enzymes in biocontrol are further supported by many researches [8, 9, 10, 11, 12,13]. [12] Pointed out that hyphae of the biocontrol agent penetrated into infected epidermal and cortical tissue of the root to destroy the hyphae of the pathogen, with a little or no damage to uninfected plant tissue. The author ascribed this biocontrol phenomenon to production of endo- and exo-chitinase by the antagonist.

**Table (1). Activity of chitinases in different FBA / pathogen interactions.**

| FBA's                     | Chitinase activity (Unit / ml) |                        |                   |                      |                  |                   | Control                   |      | LSD |
|---------------------------|--------------------------------|------------------------|-------------------|----------------------|------------------|-------------------|---------------------------|------|-----|
|                           | <i>R. solani</i>               | <i>S. sclerotiorum</i> | <i>P. ultimum</i> | <i>M. phaseolina</i> | <i>F. solani</i> | PD without chitin | PD amended with chitin 2% |      |     |
|                           |                                |                        |                   |                      |                  |                   |                           |      |     |
| <i>T. harzianum</i>       | 50.98                          | 56.84                  | 2.02              | 59.89                | 59.12            | 1.88              | 44.96                     | 6.05 |     |
| <i>T. viride</i>          | 48.66                          | 52.18                  | 1.84              | 54.00                | 54.14            | 0.00              | 19.42                     | 4.97 |     |
| <i>T. koningii</i>        | 39.10                          | 40.93                  | 3.21              | 44.18                | 44.79            | 3.52              | 13.53                     | 4.51 |     |
| <i>T. aureoviride</i>     | 36.15                          | 38.40                  | 1.68              | 40.17                | 42.35            | 0.00              | 8.58                      | 4.42 |     |
| <i>T. longibrachiatum</i> | 25.75                          | 29.52                  | 3.29              | 30.32                | 31.82            | 3.05              | 7.53                      | 3.95 |     |
| <i>G. roseum</i>          | 10.00                          | 10.23                  | 5.02              | 10.08                | 12.22            | 4.44              | 4.50                      | 3.15 |     |
| LSD                       | 4.32                           | 4.87                   | 1.69              | 6.17                 | 6.19             | 1.49              | 2.32                      |      |     |



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