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Chitinase production ability of *Trichoderma harzianum* isolates by Turbidity method

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Abstract
Fungi in the genus *Trichoderma* are among the most promising biocontrol agents against plant-pathogenic fungi. Specific strain have ability to control a range of pathogens under a variety of environmental conditions. In this study, total five isolates of *Trichoderma harzianum* were studied. Three were isolated from rhizosphere soil of different places i.e., TH2, TH3, TH4 and two isolates procured from different places i.e., TH1 and TH5. Recently, a few chitinolytic enzymes from *T. harzianum* Rifai were purified and characterized, the availability of purified chitinolytic enzymes from Trichoderma permits studies on their effects against other fungi. The data presented in table 1, showed that TH1 (T_1) content maximum i.e. 0.65 chitinase enzyme units/ mg of protein units.

Keywords: *Trichoderma harzianum*, chitinase production

Introduction
Fungi in the genus *Trichoderma* are among the most promising biocontrol agents against plant-pathogenic fungi. Specific strain have ability to control a range of pathogens under a variety of environmental conditions (Papavizas, 1985) [5]. Moreover, they may be rhizosphere competent, which allows them to colonize and protect plant roots. Their biological activity can be increased by genetic manipulation Harman *et al*. 1993, Hayes 1993 [9].
In spite of extensive research on utilization of these fungi for biocontrol, the mechanisms by which they control plant pathogenic fungi are not understood. Among the mechanisms proposed is mycoparasitism. It is presumed that this complex process requires the production of enzymes that digest the fungal cell wall Chet, 1987 [7]. *Trichoderma* spp. are known to be efficient producers of polysaccharides lyses, proteases and lipases, all of which may be involved in cell wall degradation Cherif and Benhamou 1990, Harman *et al*. 1993 [8, 9]. Among the enzymes most commonly suggested to be involved in mycoparasitism are those that degrade chitin. Recently, a few chitinolytic enzymes from *T. harzianum* Rifai were purified and characterized Harman *et al*. 1993 [9]. The availability of purified chitinolytic enzymes from Trichoderma permits studies on their effects against other fungi. Therefore, the present study was planned to with the objective of procurement and isolating *Trichoderma harzianum* from rhizosphere soil of different places and to check Chitinase production ability of *Trichoderma harzianum* isolates by Turbidity method.

Materials and Methods
Procurement and isolation of *Trichoderma harzianum* from rhizosphere soil samples
In this study, total five isolates of *Trichoderma harzianum* were studied. Three were isolated from rhizosphere soil of different places i.e., TH2 (Jabalpur M.P), TH3 (College of Agriculture, Nagpur), TH4 (Dapoli, Konkan, M.H.) And two were procured from different places i.e., TH1 (CICR, Nagpur) and TH5 (College of Agriculture, Nagpur). The dilution no. 10-4 to 10-5 harvested a good no. of fungal colonies after 7 days of incubation. The *Trichoderma harzianum* colonies were picked up from the Petri plates and placed on fresh PDA plates for obtaining pure culture. Through frequent sub-culturing the test fungus was purified and its pure culture maintained at 4 °C on PDA slants for further studies.

Estimation of chitinase enzyme

The estimation of chitinase enzyme in effective isolates was done by method suggested by Kulkarni and Ramanujam *et al*. (2010) [6]. *Trichoderma harzianum* isolates were grown on synthetic media (Czapek's broth) along with crab shell chitin (50 ml in 250 ml flask). After inoculating with 5 x 20^6 / ml conidia, these flasks were kept on rotary shaker at 140 rpm at 25 °C for 4-5 days. Culture filtrate was collected after separating the biomass filtered with nylon cloth and dialyzed with 50 mm potassium phosphate buffer pH 6.7 (6: 1) at 40 °C overnight. Sodium azide was added to keep it for further usage.

Turbidity method

The endochitinase activity was measured by the reduction of turbidity of a suspension of colloidal chitin. A suspension containing 1% (w/v) or moist colloidal chitin was prepared in 50 mm potassium phosphate buffer, pH 6.7. A mixture consisting of 0.5 ml each of chitin suspension and the enzyme solution to be tested was prepared and inculcated for 24 h at 30 °C. Subsequently the mixture was diluted with 5 ml and the optical density was read at 510 nm. Enzyme activity was calculated as the percentage of reduction of a chitin suspension by 5 percent

Preparation of colloidal chitin

Colloidal chitin was prepared as per the method of Roberts and Selintrenikoff (1988) [12].

- 5 g of chitin powder (HI Media Laboratories Pvt. Ltd., Mumbai) was added slowly into 60 ml of concentrated HCl (Sd. Fine Chemicals Ltd., Mumbai) and left for vigorous shaking overnight at 4 °C.
- The mixture was added to two liters of ice-cold 95 percent ethanol with rapid stirring and kept overnight at room temperature (25 °C).
- The precipitate was collected by centrifugation at 5,000 rpm for 20 minutes at 4 °C and then washed with sterile distilled water until the pH of the colloidal chitin turned neutral (pH 7.0).
- Later, colloidal chitin solution (5 percent) was prepared and stored at 4 °C for further use.

Preparation of phosphate buffer (pH 6.7)

- Potassium dihydrogen phosphate (KH_2PO_4 1 M) 136 g in 1000 ml of distilled water was mixed.
- Potassium hypophosphate (K_2 HPO_4) (1 M) 174 g in 1000 ml of distilled water. Both were mixed and dilute up to required concentration (50 ml) and pH should be maintained 6.7.

Preparation of standard graph

The standard graph was constructed by using dextrose ('AR' grade) as glucose source. Standard solutions of glucose (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 %) were prepared. 0.5 ml of each standard solution and chitin suspension were mixed in test tubes and incubated for 24 hr. at 30 °C

The absorbance at 510 nm was recorded using systronics make spectrophotometer after dilution with 5 ml distilled water.

Chitinase enzyme activity of *Trichoderma harzianum* isolates by turbidity method

The data presented in table 1, showed that TH1 (T_1) content maximum i.e. 0.65 chitinase enzyme units/ mg of protein units followed by TH2 (T_2), TH3 (T_3) and TH5 (T_5)) i.e., 0.64, 0.61 and 0.60 unit/mg of protein. The isolate TH4 (T_4) contain 0.36 lowest chitinase enzyme units/ mg of protein.

Table 1: Chitinase enzyme activity of *Trichoderma harzianum* isolates by turbidity method

Tr. No.	Treatment	Chitinase enzyme (unit/mg of protein)
T_1	TH1 (CTH)	0.65
T_2	TH2 (BTH)	0.64
T_3	TH3 (STH)	0.61
T_4	TH4 (ATH)	0.56
T_5	TH5 (VTH)	0.60
T_6	Control	0.36
	SEm ±	0.0005
	CD (P=0.1)	0.0020

Similar result confirmed with findings Mhaske *et al.* (2019) [1] The *Trichoderma* isolates were assayed for estimation of chitinase enzyme and among the 14 isolates, TrND-14 (Nandura) found to possessed highest chitinase enzyme i.e., 0.65 units/mg protein. Agrawal and Kothasthani (2012) [2] also studied the chitinase activity of *Trichoderma* on CCA medium. Chitin is second most abundant polymer in nature after cellulose and play major role in fungal cell wall. K. K. Suryawanshi *et al.* (2013) [3] obtained twenty four *T. viride mutants* by chemical mutagenesis i.e. Ethyl methyl sulphonate (EMS) and Hydroxyl amine (HA) treatments of different doses and time variables. Among these twenty four mutants, *T. viride* TVME3c, TVME4c, TVME4a showed maximum antagonistic activity against the *S. rolfsii*, *R. bataticola* and *F. solani*, and also having the highest chitinase enzyme units/mg of protein i.e. 0.62, 0.63 and 0.61 enzyme units/mg of protein. Savita *et al.* (2015) [4] studied sixteen *T. viride* mutants by gamma irradiation and tested for their antagonistic activities *in vitro.* Morphological characters of efficient mutants were tested up to six generation to check their stability. Among these TVGM1 were proved as effective antagonists against *S. rolfsii, R. bataticola* and *F. oxysporum* f.sp. *ciceri* basis of their maximum bio efficiency. The highest chitinase enzyme units/mg of protein i.e. 0.62 was exerted in TVGM1.

Conclusion

In conclusion, this study focused on the procurement and isolation of *Trichoderma harzianum* from various rhizosphere soil samples and assessed their chitinase enzyme activity. Five isolates of *Trichoderma harzianum* were examined, originating from different geographical locations and environments. Through detailed cultivation and purification processes, these isolates were evaluated for their ability to produce chitinase, a crucial enzyme involved in fungal cell wall degradation. The results, quantified using the turbidity method, revealed varying levels of chitinase activity among the isolates, with TH1 demonstrating the highest activity followed closely by TH2, TH3, and TH5, while TH4 exhibited the lowest activity. These findings underscore the potential of *Trichoderma harzianum* isolates in agricultural and biotechnological applications, particularly in biocontrol and enhancing plant growth by enzymatic degradation of chitin, a significant component of fungal cell walls. Further research into the genetic and biochemical mechanisms underlying chitinase production in these isolates could unveil novel insights for sustainable agricultural practices and biotechnological advancements.

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