# Efficacy of *Trichoderma* spp. in cellulase enzyme complex production

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**SUMMARY**

The present study was carried out to analyze the production of cellulase enzyme complex like endo-β 1,4 glucanases ($C_x$ cellulase) and exo-β 1,4 glucanases($C_1$ cellulase) at extracellular and intracellular level at different concentration of carbon and nitrogen sources by cellulolytic fungi *Trichoderma viride,T.koningii and T.harzianum.*A significant increase in extracellular endo-b 1,4 glucanase activity was recorded in *T. viride* in 2 % glucose as carbon source (48.63 $Ul^{-1}$) and 91.76 $Ul^{-1}$ in 3 % urea as nitrogen source and maximum intracellular endoglucanase activity was registered by *T. viride* 33.60 $Ul^{-1}$ in 3 % glucose as carbon source and 96.46 $Ul^{-1}$ in 3 % urea as nitrogen source. *T. viride* showed maximum exoglucanase activity of 45.86$Ul^{-1}$ at 3 % concentration of maltose as carbon source and 86.73 $Ul^{-1}$ at 3 % of urea as nitrogen source at extracellular level. A significant increase in exoglucanase activity was registered by *T. viride* in 3 % glucose as carbon source (32.66$Ul^{-1}$) and 3 % urea as nitrogen source (119.86$Ul^{-1}$) at intracellular level when compared to the other fungal samples.

**Key words :** *Trichoderma* spp., Endoglucanase, Exoglucanase

Cellulose occurs as the structural element of plants and is thus present as a major component in agricultural and municipal waste and is the earth's most abundant renewable resource. Two main obstacles hindering the efficient transformation of cellulose are the highly ordered crystalline cellulose structure and a lignin seal usually surrounding cellulose fibers.Cellulose is commonly degraded by an enzyme called cellulase.The cellulase is a complex system comprised mainly of three enzymes endo-β-glucanase, exocellobiohydrolase and β-glucosidase. These enzymes comprise together a system to convert cellulose to glucose.Flilamentous fungi particularly *Aspergillus* spp. and *Trichoderma* spp. are efficient producers of cellulase enzyme complex,which degrades cellulose into soluble sugar glucose (Peij *et al*.,1998).Cellulase synthesis has been shown to be affected by various kinds of carbon and nitrogen sources , pH and surface active substances(Desai and Patel,1982).

Hence, the present investigation was carried out to analyze the efficacy of *Trichoderma* spp. (*T. viride,T. koningii and T. harzianum*)in the production of cellulase enzyme complex like endo-β 1,4 glucanases($C_x$ - cellulase) and exo-β 1,4 glucanases($C_1$-cellulase) at extracellular and intracellular level at different concentration of carbon and nitrogen sources.

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## MATERIALS AND METHODS

*T.viride, T.koningii and T.harzianum* were bought from Institute of Microbial Technology, Chandigarh, India. Fresh spent mushroom substrate samples were collected from Tamil Nadu Agricultural University, Coimbatore.

***Growth medium for fungal culture:***

*Potato dextrose agar medium (PDA)*- (Riker and Riker, 1936)*:*

From peeled potato (250 g), PDA medium was prepared by adding dextrose (20 g) and agar (15 g) .The volume was made into 1000 ml with distilled water and sterilized. The medium was poured into sterilized Petriplates (15 ml/ plate).Under aseptic conditions, *Trichoderma* spp. were inoculated. The bacterial growth was suppressed by the addition of 1ml of 10,000 ppm streptomycin sulphate solution and growth pattern was noticed.

***Enzymology:***

*Preparation of culture medium:*

*Czapex-dox liquid medium(Raper and Thom, 1949)*

Czapek-dox liquid medium was prepared by adding cellulose( 10g ), sodium nitrate(2.0 g),potassium chloride (0.5 g),magnesium sulphate (0.5 g), dipotassium hydrogen phosphate (1.0 g), ferrous sulphate (0.01 g) in 1000 ml distilled water with 1.0 ml of trace metal solution comprising of zinc sulphate and copper sulphate (1.0 g and 0.5 g dissolved in 100 ml distilled water).About fifty ml of Czpek- dox liquid medium was dispensed in 250ml Erlenmeyer flasks and sterilized at 1 atm for 15 minutes. After cooling, one ml of streptomycin sulphate (10,000 ppm) was added. The pH of the medium was maintained

at 6.5 after sterilization.

The fungal species *Trichoderma viride, T.koningii and T.harzianum* grown on PDA medium for five days in Petridishes were taken with the help of sterilized cork borer (10mm dia) and were inoculated to the liquid medium. The flasks were incubated for 5-7 days. Cellulolytic fungi, *T.viride, T.koningii and T.harzianum* were assayed for their cellulase enzyme complex activity like endo-β 1,4 glucanases and exo-β 1,4 glucanases in the culture filtrate of the fungi (extracellular) and also in the mycelium (intracellular).

***Preparation of culture filtrate as enzyme source(Extracellular):***

The mycelium was filtered through Whatman No.40 filter paper using a Buchner funnel under suction and the clear filtrate was used as the source of enzyme (extracellular enzyme).

***Preparation of cell free enzyme from fungal mycelium (Intra cellular):***

The fungal mycelium was washed with distilled water twice. A quantity of one gram of the washed mycelial mat was macerated in five ml of sodium acetate buffer, pH 5.2 for endoglucanase assay and five ml of Sodium citrate buffer,pH 5.0 for exoglucanase assay in a pre chilled motar and pestle. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 x g for 15 minutes. The supernatant served as enzyme source.

***Extimation of endo- -1, 4 glucanase ($C_x$ – cellulase) (Mahadevan and Sridhar, 1982):***

About 4.0 ml of 0.5 per cent CMC solution, 1 ml of Sodium acetate–acetic acid buffer and 2ml of enzyme extract were pipetted out in a test tube and incubated in water bath at $30^0$ C. Withdrawn aliquots of 1 ml from each tube at pre- fixed interval. After that 0.5 ml of DNS reagent was added and heated in a boiling water bath for 5 min. While the tubes were warm, 1.0ml of potassium sodium tartrate solution was added and cooled to room temperature. The volume was made upto 5ml with water. The absorbance was measured at 540nm.A standard graph was prepared with glucose in the concentration range of 50µg/ml.The enzyme activity was expressed as the mg glucose released $min^{-1}mg^{-1}$ protein. Protein content of the enzyme was determined following the method of Lowry *et al*. (1951).

***Extimation of exo- -1, 4 glucanase ($C_1$ – cellulase) (Sadasivam and Manikam, 1996):***

About 0.45ml of one per cent CMC solution and 0.5ml of enzyme extract were pipetted out in a test tube and incubated at $55^0$ C for 15 min. After that 0.5 ml of DNS reagent was added and heated in a boiling water bath for 5 min.While the tubes were warm, 1.0ml of potassium sodium tartrate solution was added and cooled to room temperature. The volume was made upto 5ml with water. The absorbance was measured at 540nm.A standard graph was prepared with glucose in the concentration range of 50µg/ml.The enzyme activity was expressed as the mg glucose released $min^{-1}mg^{-1}$ protein. Protein content of the enzyme was determined following the method of Lowry *et al*. (1951).

## RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

***Endo- -1, 4 glucanase ($C_x$ – cellulase) at extracellular level:***

The fungus, *Trichoderma viride* registered a high level of endoglucanase activity of 48.63 $Ul^{-1}$ at extracellular level in 2 % glucose followed by *T. koningii*, 39.23 $Ul^{-1}$ in 1% glucose with the controls showing least activity and the minimum activity of 13.66 $Ul^{-1}$was registered in *T. harzianum* in 1% maltose as carbon source. Among the nitrogen sources, the endoglucanase activity was expressed at a higher level of 91.76 $Ul^{-1}$ in *T. viride*, in 3 % urea followed by *T. koningii* ,43.43 $Ul^{-1}$ in 1% ammonium sulphate with the controls showing least activity and the minimum activity of 13.2 $Ul^{-1}$ was registered in *T. harzianum* in 1% ammonium sulphate.

***At intracellular level:***

The endoglucanase activity at intracellular level was very much pronounced in *Trichoderma viride*, 33.60 $Ul^{-1}$ and in *T.koningii*, 33.36 $Ul^{-1}$ in 3 % glucose as the carbon source. Among the nitrogen sources, the maximum endoglucanase activity of 96.46 $Ul^{-1}$ was recorded in *T. viride* followed by *T. koningii* 95.20 $Ul^{-1}$ in 3 % urea with very low expression in controls.

The endoglucanase activity was recorded minimally as 16.43$Ul^{-1}$ in *T.harzianum*, in 2 % glucose as carbon source and 17.63 $Ul^{-1}$ in 3 % ammonium sulphate as nitrogen source.

Similar result was reported by Turpeinen *et al. (*2005) who investigated the endoglucanase activity in several solid and liquid media of *Paecilomyces inflatus* isolated from municipal waste compost. All carbon sources supported the growth of *Paecilomyces inflatus* as well as enhanced endoglucanase activity.

**Table 1 : Endo- 1, 4 glucanase activity at extracellular and intracellular level**

| Treatments | Concentration (In per cent) | Extra cellular level | | | | | Intracellular level | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbon source | | Nitrogen source | | | Carbon source | | Nitrogen source | |
| | | Control | Glucose | Maltose | Ammonium sulphate | Urea | Control | Glucose | Maltose | Ammonium sulphate | Urea |
| *Trichoderma* | 1 | 15.76 | 38.33 | 25.36 | 31.83 | 58.66 | 31.73 | 27.63 | 29.06 | 30.50 | 56.43 |
| *viride* | 2 | | 48.63 | 34.76 | 38.30 | 75.23 | | 31.93 | 30.30 | 36.60 | 72.63 |
| | 3 | | 37.36 | 48.13 | 59.06 | 91.76 | | 33.60 | 32.70 | 39.26 | 96.46 |
| *T.koningii* | 1 | 25.96 | 39.23 | 12.86 | 43.43 | 36.20 | 35.40 | 23.73 | 25.40 | 74.40 | 53.03 |
| | 2 | | 28.16 | 15.40 | 38.70 | 37.76 | | 29.40 | 26.96 | 31.00 | 54.43 |
| | 3 | | 14.00 | 26.26 | 28.33 | 38.46 | | 33.36 | 28.03 | 29.26 | 95.20 |
| *T.harzianum* | 1 | 15.60 | 18.76 | 13.66 | 12.93 | 17.40 | 24.23 | 23.16 | 29.56 | 23.56 | 28.33 |
| | 2 | | 21.90 | 17.60 | 17.30 | 23.30 | | 16.43 | 19.20 | 19.06 | 26.16 |
| | 3 | | 38.80 | 18.53 | 21.30 | 26.83 | | 22.03 | 17.36 | 17.63 | 25.53 |
| | | S.E.± | 0.16 | | 0.14 | | 0.14 | | | 0.06 | |
| | | C.D. (P=0.05) | 0.44 (P < 0.01) | | 0.39(P < 0.01) | | 0.38 (P < 0.01) | | | 0.16(P < 0.01) | |

*Enzyme activity expressed in $Ul^{-1}$ $mg^{-1}$ enzyme protein.

**Table 2 : Exo- 1, 4 glucanase activity at extracellular and intracellular level**

| Treatments | Concentration (In per cent) | Extracellular level | | | | | Intracellular level | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbon source | | Nitrogen source | | | Carbon source | | Nitrogen source | |
| | | Control | Glucose | Maltose | Ammonium sulphate | Urea | Control | Glucose | Maltose | Ammonium sulphate | Urea |
| *Trichoderma* | 1 | 28.00 | 33.96 | 36.33 | 44.06 | 62.03 | 24.66 | 25.90 | 19.44 | 30.13 | 44.53 |
| *viride* | 2 | | 41.0 | 41.36 | 42.06 | 77.63 | | 30.20 | 27.90 | 34.43 | 67.83 |
| | 3 | | 42.73 | 45.86 | 45.86 | 86.73 | | 32.66 | 29.66 | 43.33 | 119.86 |
| *T.koningii* | 1 | 22.00 | 22.46 | 31.26 | 18.83 | 25.10 | 34.50 | 21.43 | 19.33 | 34.30 | 50.40 |
| | 2 | | 25.96 | 38.70 | 19.93 | 27.00 | | 23.03 | 23.63 | 35.53 | 54.46 |
| | 3 | | 38.20 | 45.53 | 23.86 | 29.76 | | 21.33 | 25.50 | 22.43 | 74.10 |
| *T.harzianum* | 1 | | 24.03 | 13.0 | 11.20 | 17.90 | | 23.50 | 12.26 | 20.46 | 22.00 |
| | 2 | | 29.76 | 17.26 | 39.66 | 21.66 | | 24.76 | 17.33 | 23.50 | 23.90 |
| | 3 | 23.06 | 24.03 | 13.13 | 18.30 | 30.03 | 23.43 | 20.80 | 23.46 | 17.33 | 27.00 |
| | | S.E.± | 0.25 | | 0.17 | | | 0.06 | | 0.09 | |
| | | C.D. (P=0.05) | 0.67 (P < 0.01) | | 0.46(P < 0.01) | | | 0.18 (P < 0.01) | | 0.25 (P < 0.01) | |

*Enzyme activity expressed in $Ul^{-1}$ $mg^{-1}$ enzyme protein.

***Exo- -1, 4 glucanase ($C_1$ – cellulase) at extracellular level:***

Extracellular exoglucanase activity was expressed maximally 45.86 $Ul^{-1}$ in *Trichoderma viride*, 45.53 $Ul^{-1}$ in *T. koningii* than the controls and expressed minimally in *T. harzianum* 13.13 $Ul^{-1}$ at 3 % maltose (carbon source).Among the nitrogen sources, highest exogluconase activity was recorded in *T.viride*,86.73$Ul^{-1}$in urea (3 %) than the controls. The least exogluconase activity was observed in *T. harzianum*, 11.20 $Ul^{-1}$ in 1% ammonium sulphate.

The result is in accordance with the result of Baishya and Deka (2006) who also found an increase in exo-β-1, 4 glucanase and endo-β-1, 4 glucanase enzyme activity (1.15 $Uml^{-1}$ and 0.089$Uml^{-1}$) in glucose and (0.80 $Uml^{-1}$ and 0.093 $Uml^{-1}$) at 1% carbon source. Among the nitrogen sources peptone+ammonium sulphate showed maximum exo-β-1, 4 glucanase and endo-β-1, 4 glucanase enzyme activity of 0.78 $Uml^{-1}$ and 0.085$Uml^{-1}$.

***At intracellular level:***

A significantly higher intracellular exoglucanase activity was recorded in *Trichoderma viride*, 32.66 $Ul^{-1}$ in 3 % glucose as carbon source and 119.86 $Ul^{-1}$in 3 % urea as nitrogen source over the controls. The least activity of 12.26 $Ul^{-1}$ was observed in *T. harzianum* in 1% maltose as carbon source and 17.33 $Ul^{-1}$ in 3 % ammonium sulphate as nitrogen source .

Similar result was reported by El-Hawary and Mostafa (2001). They obtained highest levels of cellulase activities in *Trichoderma koningii* using a culture medium containing urea and ammonium sulphate together as

nitrogen sources at 0.22 per cent and 0.24 per cent. When the culture medium was supplemented either with 0.5per cent Tween 60 or Tween 80, the rate of cellulase production was increased considerably.

***Conclusion:***

The increase in endo and exo-β 1,4 glucanase enzyme activity may be due to the cellulolytic efficiency of *Trichoderma viride* and *T.koningii* .The carbon sources induce the production of cellulase, but the amount produced is variable. This is because of the influence of substrate (Carbon source) on the growth of cellulolytic organisms. Nitrogen sources played an important role in promoting enzyme activity. The cellulolytic activity of *Trichoderma viride* and *T.koningii* can be exploited for the efficient production of the cellulase enzyme complex (exo-β 1,4 glucanase and endo-β1,4 glucanase) at a commercial level which has lot of applications in industries like food, textiles, pharmaceuticals, detergents, fruit and vegetable processing.

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