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Biochemical Characterization of *Trichoderma* species Isolated from Rhizospheric Soil of U.P.

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Biocontrol agents belonging to the genus *Trichoderma* prove to be potent agents against soil borne pathogens. *Trichoderma* species not only prevents the soil borne pathogens to cause diseases but also play an important role in increasing root growth and development, crop productivity, resistance to abiotic stress and uptake and use of nutrients. During mycoparasitic action CWDEs enzyme plays a crucial role, they hydrolyze the cell wall of pathogen and ultimately released the contents of the organism. The antifungal action of *Trichoderma* includes a great variety of lytic enzymes such as chitinase, proteases, xylanase and glucanase, which play a key role in bicontrol activity of *Trichoderma*. Different carbon sources were tested for the induction of CWDEs, Out of the different carbon sources tested Birch Wood induces maximum xylanase production; CMC induces glucanase enzyme production, Twwen-80 for lipase, commercial chitin for chitinase and corn cob for cellulose induction. For xylanase and glucanase enzyme the molecular weight of these enzymes were also determined (47 & 55 Kda).

Keywords: Biochemical, *Trichoderma*, Cell wall degrading enzymes, carbon sources.

The biocontrol activity of *Trichoderma* is known since 1920s. *Trichoderma* species have both disease control and yield increase ability. *Trichoderma* species have been found to posses rhizospheric competence ability (to colonize and grow in association with plant roots). *Trichoderma* species are well known for their biocontrol ability against a wide variety of soil borne pathogens (*Macrophomina, Fusarium, Pythium* etc.) *Trichoderma* species are also well known for their ability to enhance systematic resistance in plants to control diseases as well as plant growth. *Trichoderma* employs several mechanisms against phytopathogens such as competition for nutrients, mycoparasitism, antibiotic production, production of secondary metabolites etc. Mycoparasitsm is the major biocontrol activity employed by *Trichoderma* against phytopathogens. Enzymes such as chitinase, glucanase, cellulase, xylanase and lipase produced by the fungus plays an important role in antagonism and are responsible for the suppression of the plant pathogens. These enzymes attack on the polysaccharide, chitin and β-glucan chains that are responsible for the rigidity of cell walls, thereby destroying the cell wall rigidity.

Considerable research has been made in understanding the chitinase, cellulase, xylanase and glucanase system of *Trichoderma* spp. during growth on different carbon sources. A lot of research has been made to understand the expression of chitinase and glucanase expression by various *Trichoderma* species. But the mechanism which regulates the synthesis of these

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enzymes is still not clear. It is very important to understand the process which involve in the regulation of these enzymes in order to select the most effective biocontrol strain of *Trichoderma*. *Trichoderma* spp. are widely used in agriculture as biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens (Dubey SC 2007, Papavizas GC 1985). Process of mycoparasitism involves several changes in the morphology of *Trichoderma* such as coiling and formation of appressorium-like structures, which serve to penetrate the host (McIntyre M, 2004). In the present investigation *Trichoderma* species were investigated for their ability to produce cell wall degrading enzymes.

MATERIALS AND METHODS

Isolation and characterization of *Trichoderma* isolate

Isolation was carried out from soil samples collected from different locations of an Indian state Uttar Pradesh. Isolates of *Trichoderma* species were isolated and identified in potato dextrose agar medium thereafter submitted to the Indian Type Culture Collection at IARI (Pusa, New Delhi) and allotted with specific ITCC numbers.

Production of cell wall degrading enzymes

Induction of chitinase For chitinase activity, two different insoluble chitin sources (colloidal chitin derived from commercial chitin and Seashells) were used. *Trichoderma* isolate were screened for chitinase activity and to assess the *Trichoderma* isolates spectrophotometrically for N-acetyl-²- D-glucosamine (NAGA) (for total chitinolytic activity) and p-nitrophenol (pNP) (for exochitinase activity).

Agar medium for detection of chitinase-positive microorganisms

Chitinase detection medium [comprising (per liter)0.3g MgSO4.7H2O, 3g (NH4)2SO4, 2g of KH2PO4, 1g of citric acid monohydrate, 15g agar, 200¼l Tween-80, 4.5g chitin source and 0.15g bromocresol purple; pH was adjusted to 4.7] was used for the detection of chitinase positive microorganism. Prepared media was poured into petri plates and allowed to solidify. After media solidifies 5 mm disc of the *Trichoderma* species was inoculated on the centre of petri plate and incubated at 25±2°C for seven days. After incubation process completes the plates were observed for colored zone formation (Shahidi F,Abozayoun R 2005).

Total Chitinolytic activity

For the determination of total chitinolytic activity a reaction mixture consisting of 1ml of culture supernatant, 0.3 ml of 1M sodium acetate buffer (SA- Buffer), pH 4.6 and 0.2 ml colloidal chitin was prepared and incubated for 20h at 40°C and then centrifuged at 13000 rpm for 5 min at 6 c. After centrifugation completes, 0.75 ml of the supernatant is mixed with 0.25 ml of 1% DNS reagent and 0.1 ml of 10M NaOH in a 1.5 ml of micro centrifuge tube and heated at 100°C for 5min. Absorbance of the reaction mixture was recorded atA_{582}.Chitinolytic activity was estimated in terms of the concentration (mg/ml) of NAGA released N-acetyl-β–D-glucosamine (NAGA) was used as a reference to draw standard curve.

Exochitinase activity

For exochitinase activity determination a reaction mixture containing 25 25¼l of culture filtrate, 0.2ml of pNPg solution (1mg pNPg ml-1), and 1ml of 0.1 M SA-buffer (pH 4.6) was prepared and incubated and incubated at 400C for 20h and then centrifuged at 13,000 rpm. 0.6 ml of the supernatant is mixed with 0.3 ml of .125 M Sodium tetraborate–NaOH buffer (pH 10.7) and absorbance was recorded at 400nm immediately after mixing and pNP concentration (Muzzarelli RA1997).

Induction of β-1-3 Glucanase enzyme

For the induction of glucanase enzyme by *Trichoderma* species Czapek Dox medium was used. Two carbon sources CMC and Wood dust were also supplemented in the medium at the rate of 1%. *Trichoderma* inoculated flasks were incubated for 10 days at 28°C on orbital shaker at 150 rpm. At the end of the incubation time, content of the flasks were filtered through filter papers to remove solid residues and mycelia. The obtained filtrate was centrifuged at 5000 rpm for 10 min. The obtained clear supernatant was considered as a crude enzyme source and is used for measuring enzyme activity**.** Glucanase enzyme activity was assayed using 1% (w/v) CMC as a substrate. Enzyme activity is expressed as U/mg.

For glucanase enzyme activity determination 1 ml of the enzyme solution was mixed with 1 ml of 1% CMC dissolved in 50mM

sodium acetate buffer(pH 5.0).The contents of the test tubes were mixed and incubated at 50°C for 60min. After incubation period completes the reaction was stopped by adding 3 ml DNS reagent and again incubated for 10 min. in a boiling water bath. Enzyme activity was determined at 540nm. One unit of CMCase activity was expressed as the amount of protein that liberate reducing sugar equivalent to glucose per minute under assay conditions (El-Zawahry,Y.A *et al* 2010).

Xylanase enzyme induction

Effect of different carbon sources viz. Birch Wood Xylan, Wheat Bran, Corn Cob and CMC were studied on xylanase expression. These carbon sources were added in Vogel's medium at the rate of 1%. After 5-7 days the culture mixture was taken out of orbital shaker. Supernatant obtained after centrifugation was tested for xylanase activity. Xylanase activity was assayed using 1 % (w/v) of birch wood xylan as a substrate. Reaction mixture contained 1ml of 1% xylan solution (in 0.1 M, pH 5 sodium citrate buffer) and 2ml of enzyme were added to the reaction tubes and incubated at 40°C and the amount of reducing sugar in the reaction tubes was measured using the Dinitrosalicylic Acid. After heating for 5min in a boiling water bath and cooling, the absorbance was read at 550nm using UV spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent xylose. One unit of xylanase activity is defined as 1µmol of xylose equivalent produced/min under the assay conditions (Khanna and Gauri 1993).

Induction of lipase enzyme from *Trichoderma* sp. on different carbon sources

Already reported three carbon sources (Tween 80, coconut and soybean oil) were tested for extracellular lipase synthesis. These carbon sources were supplemented in lipase production media @ 1%. The crude enzyme extract was stored at –20°C until used. Lipase activity in the synthetic media was determined titrimatrically on the basis of olive oil hydrolysis. One ml of culture supernatant was added to assay substrate, containing 10 ml of 10% (v/v) homogenized olive oil in 10% (w/v) gum acacia, 2.0 ml of 0.6% CaCl2 solution and 5 ml of phosphate buffer (pH 7.0). The enzyme substrate mixture was incubated on rotary shaker with 150 rpm at 30°C for one hour. 20 ml of alcohol: acetone (1:1) mixture was added to the reaction mixture. Liberated fatty acids were titrated with 0.1N NaOH using phenolphthalein as an indicator (Kempka *et al.,* 2008). The end point was light pink in colour. One unit of lipase is defined as "the amount of enzyme which releases one micro mole fatty acid per minute under specified assay conditions

Lipase unit calculation

$$\textit{Lipase Activity} = \frac{\Delta V \times N}{V \text{ samples}} \times \frac{1000}{60}$$

$\Delta V = V2 - V1$

V1= Volume of NaOH used against control flask

V2= Volume of NaOH used against experimental flask

N= Normality of NaOH

V (Sample) = Volume of enzyme extract

Units of extracellular lipase activity were units per ml (U mL^{-1}) while intracellular activity was calculated in units per gram (U g^{-1}).

Induction of cellulase enzyme from *Trichoderma* sp. on different carbon sources

The cultures were grown in 250 ml Erlenmeyer flask that contained 50 ml of basal salt medium (Neagu DA, 2012) .The pH of the medium was adjusted to 6.5 prior to sterilization. The flasks were inoculated with 2 agar discs (2 mm in diameter) of 7 days old culture from PDA plates and were incubated under stationary condition at 28°C up to 7 days. Mycelium was separated from the culture broth through filtration and the obtained filtrate was centrifuged at 11000 x g for 10 min to remove mycelium. The obtained supernatant is served as a crude enzyme source.

Enzyme assay

Filter paper activity (FPase) for total cellulase activity in the culture filtrate was determined according to the standard method of Hankin and Anagnostakis (Hankin L and Anagnostakis SL, 1975) . Aliquots of approximately diluted culture filtrate as enzyme source was added to whatman no. 1 filter paper strip (1 x 6 cm~ 50 mg) immersed in one milliliter of 0.05 M Sodium citrate buffer of pH 5.0. After incubation at 50 ± 2°C for 1 hrs, the reducing sugar released was determined by dinitrosalicylic acid (DNS) method (Miller GL, 1959). One unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar from filter paper per ml per min.

Endoglucanase activity (CMCase) was measured using a reaction mixture containing 1 ml of 1% carboxymethyl cellulose (CMC) in 0.5 M citrate acetate buffer (pH 5.0) and aliquots of suitably diluted filtrate. The reaction mixture was incubated at 50 ± 2°C for 1 h and the reducing sugar produced was determined by DNS method (Murao S *et al* 1988). One unit (IU) of endoglucanase activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar per min.

Endoglucanase assay

0.5 ml of the enzyme solution was added into test tubes. The enzyme and substrate solution were equilibrated at 50°C. 0.5 ml of the CMC solution was taken into the test tubes and mixed well. Incubated at 50°C for 30 min. 3.0 ml of DNS solution was added and mixed well, boiled for exactly 5.0 min in vigorously boiling water. Place the tubes in an ice-cooled water bath to quench the reaction. Add 20 ml of distilled water. Mix by inverting the tubes several times. Absorbance was taken at 540 nm. Enzyme activity is expressed as IU/ml/min.

Protein profiling of the enzymes/ isozymes isolated from *Trichoderma* sp.

The clear supernatant used as a source of crude enzyme was purified by the slow addition of Ammonium Persulfate with continuous stirring till 80% saturation. The obtained precipitate was dissolved in citrate phosphate buffer pH 5.0. Enzyme preparations were applied for FPLC treatment Sharp peak Fractions were collected and applied for SDS-PAGE analysis.

Determination of Protein Concentration

The protein content of the crude enzyme preparation was assayed by Spectrophotometer at 660nm for Lowry (1951) method.

SDS-PAGE Analysis of glucanase enzyme

Table 1. Identification of potential *Trichoderma* strains

Name of Bioagent	Culture No.	Source/ District	ITCC Acc. No.	Gen Bank NCBI No.
T. harzianum	*Th* azad	CSA Kanpur Nagar	6796	KC800922
T. viride	01PP	Hardoi	8315	JX119211
T. asperellum	T_{asp}/CSAU	CSA Kanpur Nagar	8940	KC800921
T. koningii	T_K (CSAU)	CSA Kanpur Nagar	5201	KC800923
T. atroviride	71 L	Hardoi	7445	KC008065
T. longibrachiatum	21 PP	Kaushambi	7437	JX978542
T. virens	T_{vi} (CSAU)	CSA Kanpur Nagar	4177	KC800924

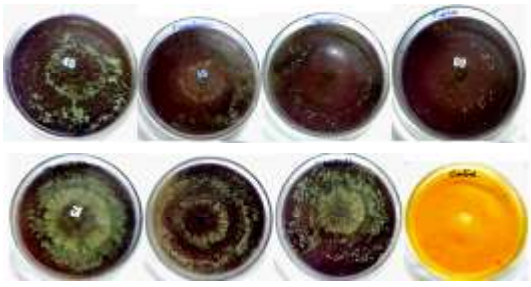


Fig. 1. Chitinase activity of *Trichoderma* species

Table 2. Spectrophotometric determination of chitinolytic and exochitinase activity of *Trichoderma* isolates in media supplemented with colloidal chitin

Isolates	Chitinolytic Activity (mg/ml)		Exochitinase activity (U/ml X 10^{-3})	
	Colloidal chitin	Seashell chitin	Colloidal chitin	Seashell chitin
T. viride	6.0	4.3	0.0125	0.00609
T. harzianum	6.2	4.8	0.0133	0.00607
T. asperellum	5.4	3.3	0.0110	0.00604
T. koningii	5.5	2	0.0097	0.0060
T. atroviride	3.9	3.3	0.0116	0.0061
T. longibrachiatum	5.6	3.1	0.0084	0.0055
T. virens	5.0	1.8	0.0076	0.0056

For molecular weight determination the enzyme preparation and known molecular weight markers were subjected to electrophoresis with 12% acrylamide gel. After electrophoresis gel was stained with Ezee blue gel stainer. Clear bands indicate the glucanase enzyme activity.

SDS-PAGE Analysis of xylanase enzyme

For molecular weight determination the enzyme preparation and known molecular weight markers were subjected to electrophoresis with 10% acrylamide gel. After electrophoresis gel was stained with Ezee blue gel stainer. Clear bands indicate the xylanase enzyme activity.

RESULTS AND DISCUSSION

The identified *Trichoderma* isolates were confirmed by morphological descriptors and deposited in Indian Type Culture Collection (ITCC), IARI, Pusa, and New Delhi (Table 1)

Production of cell wall degrading enzymes

Colloidal chitin media containing bromocresol purple (pH 4.7) when inoculated with chitinolytic *Trichoderma*, resulted in breakdown of chitin into N-acetyl glucosamine causing a drift in pH from acidic to alkaline, colour of media (BCP) changes from yellow to purple zone surrounding the inoculated fresh culture plugs in the region of chitin utilization. Chitinase activity exhibited by *Trichoderma* was determined by the diameter of the purple colored zone after 3-7 days of incubation in the colloidal chitin supplemented agar medium.

All the seven strains of *Trichoderma* tested for chitinase enzyme production have the property of secreting chitinase enzymes (Table:2 and Fig:1). The best chitinase enzyme producer is

Table 3. Xylanase activity produced by *Trichoderma* spp. grown on different carbon sources

Carbon source (1%)	*T. viride*	*T. harzianum*	*T. asperellum*	*T. koningii*	*T. atroviride*	*T. longibrachiatum*	*T. virens*
Birch Wood Xylan (BW)	5.6	5.8	5.12	5.1	5	4.3	4.19
Wheat Bran (WB)	4.2	4.4	4.7	4.1	4	3.9	3.6
Corn Cob (CC)	3.6	3.03	3	3.01	2.61	2.6	2.4
Carboxy Methyl Cellulose	3.8	3.4	3.26	3.15	3	2.9	2.5

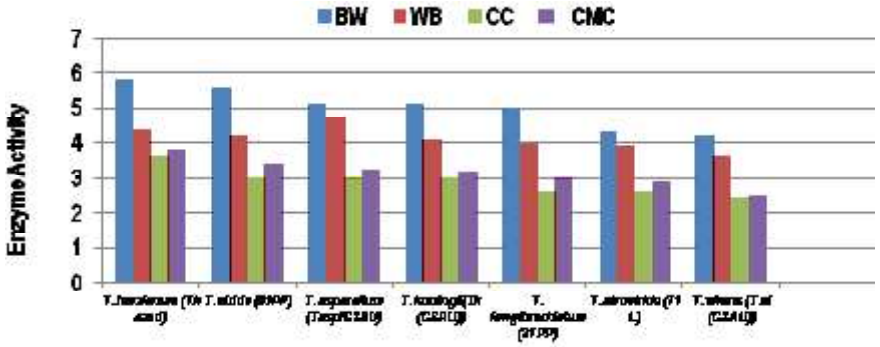


Fig. 2. Showing effect of carbon source on xylanase production by *Trichoderma* sp

Table 4. Showing glucanase activity produced by *Trichoderma* spp. grown on different carbon sources

Carbon source (1%)	*T. viride*	*T. harzianum*	*T. asperellum*	*T. koningii*	*T. atroviride*	*T. longibrachiatum*	*T. virens*
CMC	1.66	2.01	1.42	1.39	1.35	1.20	0.82
Wood DUST	0.5	0.41	0.4	0.39	0.35	0.33	0.3

T. harzianum which exhibited highest activities of both total chitinase (6.2mg/ml), seashell chitin (4.8mg/ml) and exochitinase activity (0.0133).

Although a plethora of chitinolytic enzymes have been detected and purified from various *Trichoderma* sp. (Lorito *et al.,* 1998). Many studies have proved the potential of *Trichoderma* sp. as biological agents antagonistic to several soil borne plant pathogens. The production of lytic

Enzymes influenced by the composition of the culture media (Bruce *et al.,* 1995).Maximum chitinase activity peak (0.049 IU/ml) were obtained at 62 hrs from the media containing coffee husk. In the medium containing urea, the production level of chitinase was 0.041 IU/ml. Minimum chitinase production showed when the culture grow at glycerol (0.005 IU/ml), rice bran (0.005 IU/ml) and wheat bran (0.004 IU/ml) containing medium (Bruce *et al*., 1995).

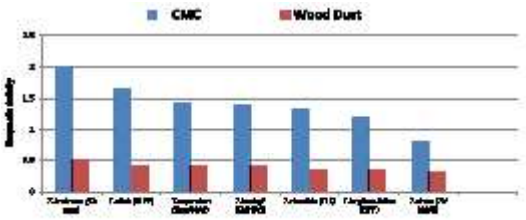


Fig. 3. Effect of carbon sources on glucanase enzyme induction

Xylanase production by *Trichoderma* species using different carbon sources was studied (Table 3 and Fig. 2). The highest xylanase enzyme induction was achieved By *Trichoderma harzianum* with Brich wood xylan (5.8). Meenkashi *et al* 2008 found maize straw as the best inducer of xylanase enzyme by *T.viride*. Irfan and Sayed 2012 2012, Kar *et al* 2013 found sugarcane baggase as the best carbon source for the induction of xylanase enzymes by *Trichoderma reesei*.

T.harzianum (2.01) and *T. viride (*1.66) produced the highest amount of glucanase enzyme. CMC found to be the best enzyme inducer (Table 4 and Fig. 3).

An exo-β-1, 3-glucanase that hydrolyzes both β-1, 3 linkages and, less efficiently, β-1, 6 linkages in *T. reesei* has also been described (Bamforth, C. W). In *T. harzianum*, the β-1, 6-glucanase activity was secreted into the growth medium containing chitin, pustulan, nigeran, fungal cell walls (De la Cruz), or autoclaved mycelia as the sole carbon source.

Data presented in Table 5 and 6, showed that cellulase production by *Trichoderma* species was significantly influenced by the type of carbon source in the basal salt medium. Corn cob was the

Table 5. Showing filter paper activity produced by *Trichoderma* spp. grown on different carbon sources

Carbon source (1%)	*T. harzianum*	*T. viride*	*T. koningii*	*T. atroviride*	*T. longibrachiatum*	*T. virens*	*T. asperellum*
Maltose	0.19	0.14	0.11	0.08	0.1	0.06	0.08
Corn Cob	1.21	1.04	0.65	0.39	0.38	0.39	0.45
Wheat Bran	0.89	0.73	0.6	0.35	0.3	0.32	0.4
Sucrose	0.45	0.3	0.25	0.12	0.13	0.1	0.16
Filter Paper	0.56	0.34	0.26	0.1	0.09	0.16	0.18

Table 6. Showing Endoglucanase activity produced by *Trichoderma* spp. grown on different carbon sources

Carbon source (1%)	*T.harzianum*	*T. viride*	*T. koningii*	*T. atroviride*	*T. longibrachiatum*	*T. virens*	*T. asperellum*
Maltose	0.23	0.16	0.15	0.1	0.07	0.07	0.09
Corn Cob	1.15	1	0.76	0.45	0.45	0.37	0.31
Wheat Bran	0.87	0.73	0.56	0.34	0.38	0.30	0.27
Sucrose	0.24	0.18	0.1	0.09	0.08	0.1	0.09
Filter Paper	0.56	0.34	0.3	0.25	0.2	0.18	0.17

Table 7. Showing lipase activity produced by *Trichoderma* spp. grown on different carbon sources

Carbon source (1%)	*T.harzianum*	*T. viride*	*T. koningii*	*T. atroviride*	*T. longibrachiatum*	*T. virens*	*T. asperellum*
Coconut oil	19.66	13.5	9.83	7.00	10.66	11.53	11.5
Sunflower oil	22.85	18.16	10.38	9.0	11.28	13.8	12.2
Tween 80	25.33	20.05	12.11	10.23	12.48	16.36	14.45

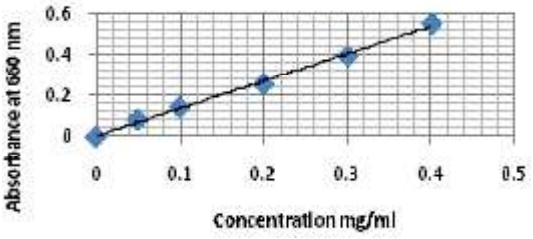


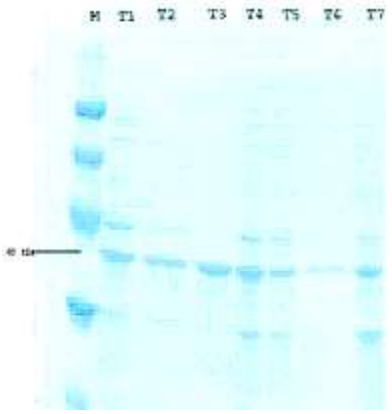
Fig. 4. Standard graph for Lowry

most effective carbon source for cellulase enzyme production followed by wheat bran, filter paper, sucrose and maltose.

Latifian *et al*., (2007) obtained the maximum cellulase activity (1.1635 U/g) using rice bran like substrate and in same conditions for incubation. Yang *et al*. (2004) found similar results for a microbial consortium of *T. reesei* (AS3.3711), *Aspergillus niger* (3.316 U/g) and *Saccharomyces*

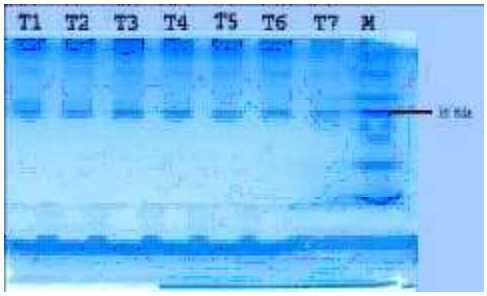
Table 8. Xylanase and Glucanase enzyme protein content of seven fungal strains

S. No.	Fungal Strain	Xylanase enzyme protein concentration (mg/ml)	Glucanase enzyme protein concentration (mg/ml)
1.	*T. harzianum*	0.21	0.23
2.	*T. viride*	0.16	0.21
3.	*T. koningii*	0.15	0.19
4.	*T. asperellum*	0.10	0.17
5.	*T. atroviride*	0.084	0.15
6.	*T. longibrachiatum*	0.07	0.12
7.	*T. virens*	0.042	0.09



(M) Molecular weight marker and (T1-T7) Activity pattern *Trichoderma* samples for determination of molecular weight. From left to right

Fig. 5 (a). 10% SDS-PAGE of xylanase enzyme



(M) Molecular weight marker and (T1-T7) Activity pattern *Trichoderma* samples for determination of molecular weight. From left to right

Fig. 5 (b). 12% SDS-PAGE of glucanase enzyme

cerevisiaes (AS2.399) on rice chaff in SSF. Similar results were also obtained using municipal solid waste residue and *Aspergillus niger*, with the maximum activity of exoglucanase (1.64U/g) and endoglucanase (1.84U/g) after 4 days (Gautam *et al*., 2011).

The production medium supplemented with different carbon sources like sunflower oil, coconut oil and tween-80 was estimated for lipase activity and the results are depicted in table 7. All strains shows maximum lipase activity in media supplemented with Tween 80. Minimum lipase activity occurs in coconut supplemented media. Among all the tested strains *T.harzianum* shows maximum lipase activity on media supplemnted with tween-80. Maximum lipase enzyme production was achieved with Tween- 80 supplemented media (Table.7).

Ohnishi *et al*., 1994 reporetd that lipase activity tended to increase as the glucose concentration increased from 0% to 4%. In another study Iftikhar *et al*., 2002, found that there was no difference between glucose and olive oil for lipase production. Moreover, the lipase activities of many other fungi, such as *Aspergillus wentii* (Chander *et al* 1980) *Mucor hiemalis* (Akhtar *et al*., 1980) (Rathi *et al* 2001) are also stimulated by the addition of glucose to the production medium.

Through Lowry method of protein quantification it is found that *T. harzianum* has the highest amount of protein (0.21 mg/ml) table 8.

For molecular weight determination, xylanase enzyme activity bands were observed at 43 kDa while in case of glucanase enzyme activity bands were observed at 55 kDa in all seven potential strains of *Trichoderma* sp (Figure 5a and 5b) .

The present work aims to investigate the production of hydrolytic enzymes on different carbon sources. it was found that hydrolytic enzymes play a crucial role in mycoparasitic action and causes inhibition of the phytopathogens (*Fusarium, Sclerotium rolfsi* etc). These results were similar to the findings of Melo Faull who reported that *T.harzianum* and *T.koningii* are effective against *R.soleni*. *Ramezani* found that *Trichoderma koningii* secretes toxic metabolites which reduce the mycelial growth of Pathogen *R.soleni*. Ramerzani also documented that *T.harzianum* is also very effective against *M. phasiolan*. Shalini and Kotasthane reported that *T. viride* and *T. harzianum* are very effective in controlling *M.pasiolinna* then *T.hamaum*. They also screened sevnteeen strains of *Trichoderma* against *R. soleni* and found that *T. harzianum*, *T. viride* and *T. aureoviride* are most effective in controlling the *Rhizoctonia*. Monterio *et al* reported that *T.harzianum* ALL42 was capable of inhibiting the growth of *R.soleni* and *M.phaseolina*, by coiling around the hyphae and formation of apressoria like structures and hooks. Almedia studied the interaction between fifteen isolates of *Trichoderma harzianum* and *R.soleni* through TEM and showed that *Trichoderma* strain effectively coils around the pathogen hyphae and produce hydrolytic enzymes. There are many reports which suggest the involvment of G proteins, cAMP and MAP kinases in the extracellular enzyme secretion and coiling around the hyphae. There are many evidences which support the involvement of G-α unit during coiling.

El- Katany *et al* showed that *T. koningi* MTCC796 elevated 1.18 fold specific activities of β-1, 3 glucanase during 72 to 96 hours as compared to 24 h incubation. It indicates that β-1, 3 glucanase inhibited the growth of pathogen in synergistic cooperation with chitinase. Marco *et al* showed that Two isolates of *T.harzianum* secretes chitinolytic enzymes and it increased within 72 h, while β-1, 3 glucanase activity was found to be maximum during 72 to 120 h. Marco *et al*. noted that two isolates of *T. harzianum* (39.1 and 1051) produced and secreted on induction of substantial amounts of chitinolytic enzymes and it increased within 72 h, while β-1, 3 glucanase activity was found to be maximum during 72 to 120 h. Growth in presence of specific substrate. There are many mechanisms which are employed by *Trichoderma* against pathogens. During antibiosis the anatgonistic fungus produce antibiotics, compete for nutrients, while in case of mycoparasitism *Trichoderma* directly attacks the pathogen by secreting CWDEs such as chitinase xylanase, glucanase protease etc. CWDEs play a crucial role in the anatagonistic mechanism as they degrade the cell wall of pathogens. These enzymes lysesed the pathogen cell wall (Sangle UR). Present findings showed higher specific activity of enzymes-chitinase, xylanase, cellulose, lipase and β-1, 3 glucanase in *Trichoderma* spp. Activity of

these enzymes varied among the *Trichoderma* species. This may be due to the expression of certain gene in *Trichoderma* spp.

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