

## Review

# Induction of chitinase, $\beta$ -glucanase, and xylanase taken from *Trichoderma* sp. on different sources: A review

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**Biological control by antagonistic organisms is a potential nonchemical tool for crop protection against phytopathogenic fungi. *Trichoderma* spp. are the most commonly used biocontrol agent that are being used against many soil borne pathogens. The biocontrol mechanism of *Trichoderma* involves mycoparasitism, antibiosis and competition for key nutrients. Chitinase, xylanase and  $\beta$ -glucanase are the major cell wall-degrading enzymes (CWDEs) that are involved in the mycoparasitic action. CWDEs improve the biocontrol activity of these strains. Different carbon sources are supplemented in the growth medium for the induction of these CWDEs.**

**Key words:** Cell wall-degrading enzymes (CWDEs), mycoparasitic, phytopathogenic, *Trichoderma*.

## INTRODUCTION

Traditional methods of crop protection include use of chemical pesticides. But the use of chemical pesticides has drastic effects on environment and consumers. Chemical pesticides are not beneficial for long term use as they pollute the environment, leave harmful residues in the soil and they also lead to the development of resistant strains. Use of biocontrol agents (BCAs) is the solution for this problem. The term biocontrol agent includes use of beneficial microbes and their products against pathogens. In present era there are number of BCAs that are available and commercialized in agriculture field against plant pathogens. To date, a number of BCAs are available as commercial products, as *Agrobacterium*, *Pseudomonas*, *Streptomyces* and

*Bacillus*, and fungal genera such as *Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida* and *Coniothyrium*. *Trichoderma* spp. are the most common soil fungi (Harman et al., 2004). These fungi are opportunistic, avirulent plant symbionts, and act as parasites and antagonists of many phytopathogenic fungi, such as *Sclerotinia*, *Pythium* etc. Thus, protecting plants from disease. *Trichoderma* spp. are among the most studied fungal BCAs and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman, 2000; Harman et al., 2004; Lorito et al., 2004). *Trichoderma* provides numerous advantages: (i) colonization of the rhizosphere by the BCA ("rhizosphere competence") allowing rapid establishment within the

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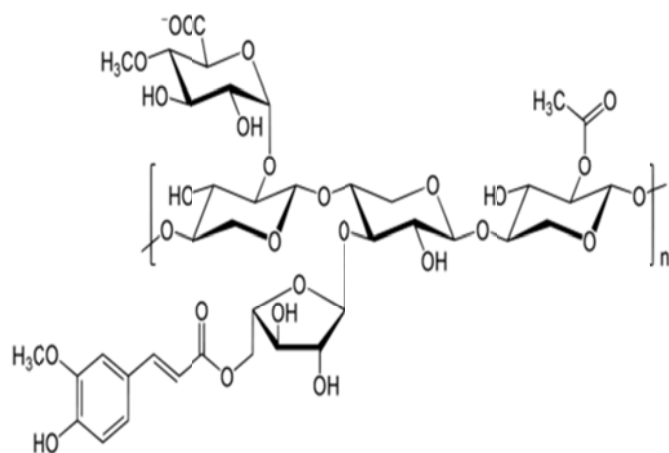


Figure 1. Structure of Xylan.

stable microbial communities in the rhizosphere; (ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms; (iii) improvement of the plant health and (iv) stimulation of root growth (Harman et al., 2004; Papavizas et al., 1985).

Several strains from the genus *Trichoderma* have been described as antagonistic fungi able to control a wide range of phytopathogenic fungi. The antifungal activity of *Trichoderma* involves production of antibiotics, including competition for key nutrients, and production of fungal cell wall-degrading enzymes (CWDEs) (Hjeljord and Tronsmo, 1998; Elad et al., 1982).

## MYCOPARASITIC ACTION AND LYTIC ENZYMES

Process of mycoparasitic action includes several steps: Recognition of the host, attack and subsequent penetration and killing. During this process *Trichoderma* secretes CWDEs that hydrolyze the cell wall of the host fungus, subsequently releasing the cell wall oligomers (Kubicek et al., 2001; Howell, 2003; Woo et al., 2006).

The antifungal action of *Trichoderma* spp. includes a great variety of lytic enzymes such as proteases, xylanase, chitinase, glucanases (Lorito, 1998; Lorito et al., 1994a, 1996a), most of which play a great role in biocontrol (Harman and Kubicek, 1998; Baek et al., 1999; Carsolio et al., 1999; Woo et al., 1999; Zeilinger et al., 1999; Kullnig et al., 2000; Kubicek et al., 2001). These CWDEs isolated from the different strains of *Trichoderma* have been purified and characterized (Lorito, 1998). Interestingly, when tested alone or in combinations, the purified proteins showed antifungal activity against a variety of pathogens (that is species of *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia* and *Colletotrichum*, as well as fungus like organisms such as the *Oomycetes Pythium* and *Phytophthora* which lack chitin in their cell walls (Tronsmo, 1991; Lorito et al.,

1993, 1994a).

The direct application of these secreted CWDEs produced by fungal BCAs, instead of using the whole "live" organisms, has numerous advantages in industry and agriculture. The selective production of these enzymes active can be increased by modifying the growth conditions, that is type and composition of culture medium, carbon sources, temperature of incubation and pH, etc. (Lorito and Scala, 1999; Lorito et al., 2006). The presence of different carbon sources, such as mono- or polysaccharides, colloidal chitin, or fungal tissues, has been shown to increase the secretion of CWDEs (Mach et al., 1999). Moreover, purified mixture of CWDEs showed improved antifungal effects against various plant pathogens (Lorito et al., 1994b, 1996a; Baek et al., 1999; Carsolio et al., 1999).

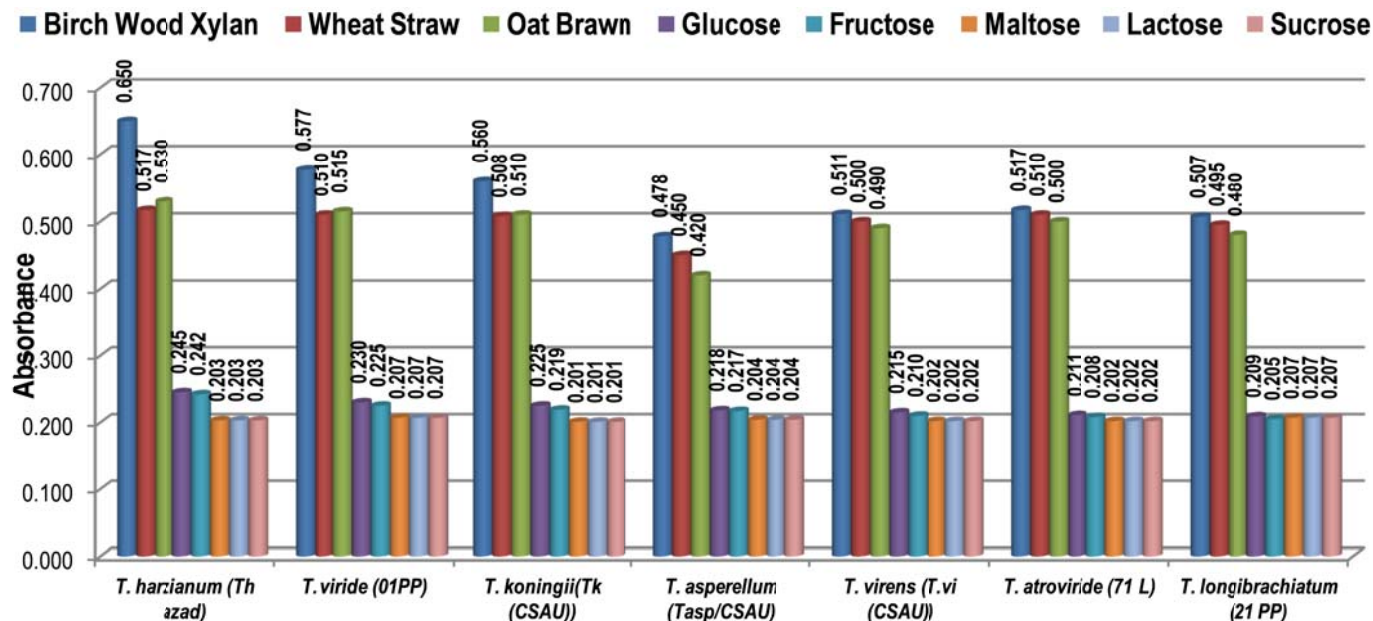
## Xylanases

Xylanases ( $\beta$ -1,4-xylan xylanohydrolase, E.C.3.2.1.8) (Figure 1) are the major components of xylanolytic enzymes and randomly cleave the  $\beta$ -1,4-glycosidic bond of xylan backbone. Xylan is the second most abundant natural polysaccharide. Xylan is a heteropolysaccharide consisting of  $\beta$ -1,4-linked D-xylose monomers in connection with side branches of arabinosyl, glucuronosyl, acetyl, uronyl, and mannosyl residues (Tsujibo et al., 1997). Complete degradation of xylan structures requires the concerted and synergistic function of several enzymes including endo- $\beta$ -1,4-xylanases (EC 3.2.1.8) (Jun et al., 2009). Due to the broad applications in biopulping and biobleaching in paper industry, xylanase has attracted the attention of many researchers (Tsujibo et al., 1997).

## Xylanase production

Solid state fermentation (SSF) was carried out at 30°C with 50 ml of Vogel's medium with some modifications and 1% of birchwood xylan in 250 ml Erlenmeyer flasks in a BOD incubator. After eight days, the contents of the flasks were filtered through filter paper and the obtained culture broths were centrifuged 10 min at 3000 × g supernatants were then assayed for extracellular xylanase activity.

For maximum xylanase production of, various culture conditions viz., carbon and nitrogen source, inoculum size, pH, temperature and incubation period were optimized by conventional "one variable at a time" approach which involves varying a single independent variable at a time while maintaining the others at a constant level. Fungal isolates were grown in different media containing 1% birchwood xylan, at pH 6.0 for 192 h. Medium giving maximum growth was further used to standardize the inoculum size by inoculating different



**Figure 1.** Effect of different carbon source on xylanase production by *Trichoderma* spp.

concentrations (1-5 ml) of inoculums. Similarly, the temperature and time of incubation were optimized by growing the fungal isolate at different temperatures (25-65°C) for different time periods (96-192 h). Different organic viz., yeast extract (YE), beef extract (BE), peptone (PPT), soybean residue (SR) and corn powder (CP) were supplemented separately to a final concentration of 0.3% (w/v) to study the microbial growth and xylanase activity. Glucose, maltose and sucrose at a final concentration of 0.1% and wheat bran (WB), corn cob (CC) birchwood xylan (BW) and carboxy methyl cellulose (CMC) were used as the carbon source at a final concentration of 1.0% (Figure 2). After 7 days of growth, the fungal broth was filtered through filter paper and centrifuged at  $3000 \times g$  for 15 min at 4°C in a refrigerated centrifuge. The supernatant taken as enzyme extract containing extracellular xylanase was used to assay the enzyme activity. The highest xylanase activity was shown by Birch wood xylan (Sonika et al., 2014)

### Applications of xylanases

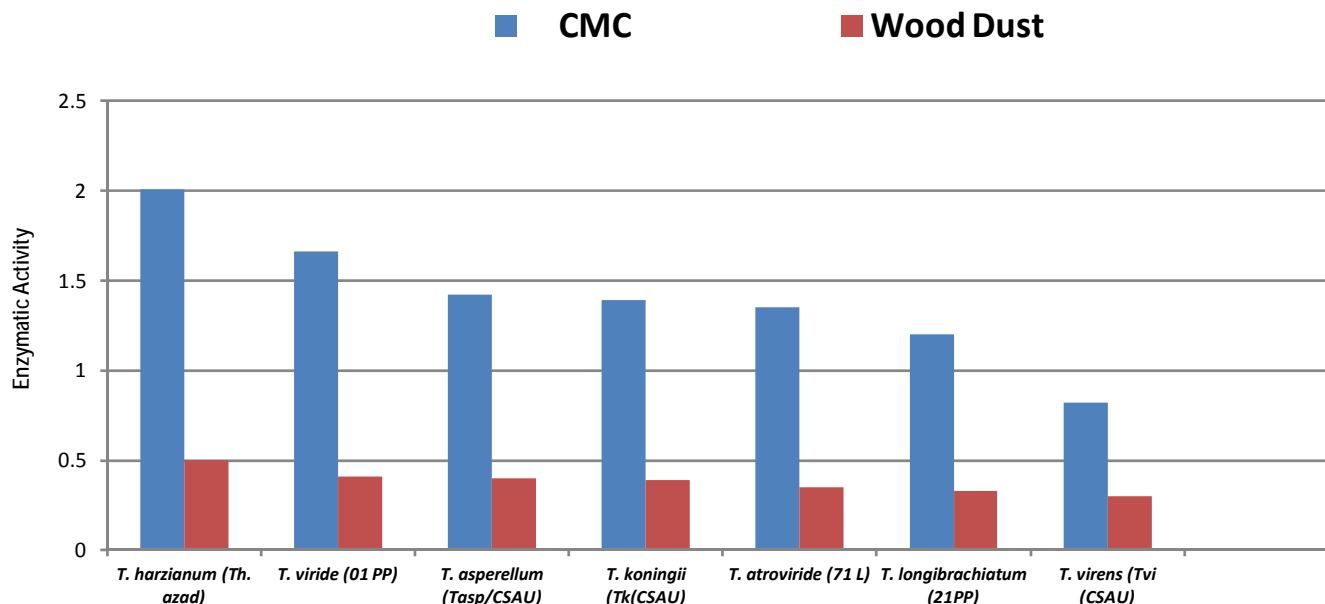
Besides the use of xylanase enzymes in antagonistic mechanism, the potential applications of xylanases also include the bioconversion of lignocellulosic material and agro-wastes into fermentative products, the clarification of juices, the improvement of the consistency of beer and the digestibility of animal feed stocks. The incorporation of xylanase from *Trichoderma longibrachiatum* into the rye-based diet of broiler chickens reduced intestinal viscosity, thus, improving both the weight gain of the chicks and their feed conversion efficiency. One of the most important biotechnological applications of xylanase

is its use in pulp bleaching.

### Glucanase enzyme

$\beta$ -1,3-glucanases are hydrolytic enzymes, widely distributed among bacteria, fungi and higher plants.  $\beta$ -1,3(1,6)-glucans are major components in cell wall of yeasts and fungi. In most yeast, especially *Saccharomyces*. *Trichoderma* spp. secretes  $\beta$ -1,3-glucanases when it is grown on polysaccharides, fungal cell walls.

Cellulose is the most abundant organic polymer on earth. Cellulases hydrolyze  $\beta$ -1,4- glycosidic bond in the cellulose structure and operate as a multicomponent enzyme system which consists of three classes of enzyme; Endoglucanases (endo- $\beta$ -1,4-glucanase, CMCCase, EC 3.2.1.4), cellobiohydrolases (exoglucanase, avicelase, EC 3.2.1.91) and  $\beta$ -glucosidase (cellobiase, EC 3.2.1.21) (Dincer and Telefoncu, 2006). The first two enzymes are called as "real cellulose" and produce cellobiose and glucose by acting directly on cellulose. The cellobiose is then hydrolyzed into glucose by  $\beta$ -glucosidase (Dincer and Telefoncu, 2006; Andersen, 2007). Endoglucanases cleave the internal glycosidic bonds of cellulose chains and act synergistically with exoglucanase and  $\beta$ -glucosidase during the hydrolysis of crystalline cellulose. Also, endoglucanases are used in increasing the yield of fruit juices, beer filtration, and oil extraction, improving the nutritive quality of bakery products and animal feed, and enhancing the brightness, smoothness, and over all quality of cellulosic garments. Therefore, new fungal



**Figure 3.** The effect of carbon sources on glucanase enzyme induction.

sources and properties (optimal pH and temperature) of EG must be investigated (Naika et al., 2007).

### Glucanase enzyme production

*Trichoderma* sp. previously isolated from the different states of Uttar Pradesh were cultivated on Czapek Dox Medium containing CMC and wood dust as sole carbon source (1%) (Figure 3). Cultures were incubated for 10-14 days on orbital shaker at 150 rpm. At the end of the incubation time contents of conical flasks were filtered and the filtrate was centrifuged at 5000 rpm for 10 min. The clear supernatant was considered as the source of crude enzymes. 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 and used for enzyme activity determination (Pandey et al., 2014).

### Chitinase enzyme

*Trichoderma* are well known producer of chitinolytic enzymes and used commercially as a source of these proteins. Due to the importance of chitinolytic enzymes in insect, nematode, and fungal growth and development, they are receiving attention in regard to their development as biopesticides or chemical defense proteins in transgenic plants and microbial biocontrol agents. In this sense, biological control of some soil-borne fungal diseases has been correlated with chitinase production.

Fungi and bacteria producing chitinases exhibit antagonism against fungi, and inhibition of fungal growth by plant chitinases has been demonstrated. Insect pathogenic fungi have considerable potential for the biological control of insect pests.

Chitin, a homopolymer of  $\beta$ -(1, 4)-N-acetylglucosamine (GlcNAc) is the second most abundant source of nutrients and energy after cellulose (Agrawal and Kotasthane, 2012; Lorito et al., 2004; Tronsmo, 1991; Dincer and Telefoncu, 2006). It is widely distributed in the nature as the integuments of insects and crustaceans and as a component of fungi and algae (Baek et al., 1999; Hjeljord and Tronsmo, 1998). Chitinases are a group of enzymes that decompose chitin into a variety of products that include the deacylated oligomer chitosan, the disaccharide chitobiose and the monomer N-acetyl glucosamine (Dincer and Telefoncu, 2006; Pandey et al., 2014). The present study was aimed for screening the different *Trichoderma* species to select potent isolate, production and purification of chitinase followed by determination of its molecular weight (Saraswathi and Jaya, 2014).

For the evaluation of chitinases activity, two different insoluble chitin sources (colloidal chitin derived from commercial chitin and Seashells) were used. Chitinase detection medium consisted of a basal medium comprising (per liter) 0.3 g of  $MgSO_4 \cdot 7H_2O$ , 3.0 g of  $(NH_4)_2SO_4$ , 2.0 g of  $KH_2PO_4$ , 1.0 g of citric acid monohydrate, 15 g of agar, 200  $\mu$ l of Tween-80, 4.5 g of chitin source and 0.15 g of bromocresol purple; pH used was adjusted to 4.7. Lukewarm medium was poured in Petri plates and allowed to solidify. Fresh culture plugs of the isolates to be tested for chitinase activity were inocu-

lated on the medium and incubated at 25±2°C and were observed for colored zone formation (Agrawal and Kotasthane, 2012). In our study, it was found that during the study the colloidal chitin derived from the commercial chitin was more potent in inducing chitinase enzyme activity as compared to the colloidal chitin derived from the sea- shells.

### Conflict of Interests

The authors have declared that they have no conflict of interests.

### ACKNOWLEDGEMENT

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