# SCREENING OF TRICHODERMA SP. STRAINS FOR PRODUCING HYDROLITIC ENZYMES

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**Abstract:** Besides other mechanisms of action, producing of lytic enzymes such as chitinases, glucanases, proteases, lipases have been associated with the ability of *Trichoderma* spp. to control pathogens. The abillty to produce lytic enzymes is a widespread property of rhizosphere fungi. The aim of this study was the screening of four *Trichoderma* strains for lytic enzymes production. Strains were tested to find their ability to produce chitinases and cellulose by plate method. The results obtained in this study using screening plate method demonstrated that all strains of *Trichoderma* studied are cellulase and chitinases producing. The Td85, Td50 strains had a medium enzymatic index and Tal12 and Td49 showed the highest enzymatic index for cellulolitic activity. The Td49 and Tal12 strains showed a large purple color zones with higher chitinase activity as compared to Td50 strain that show low chitinase activity.

**Keywords:** *cellulase, chitinase, mycoparasitism*

## INTRODUCTION

For a long time, *Trichoderma* species have been known as biological agents for control of plant diseases. They produce and release many components which induce local or systemic plant resistance to abiotic stress (Harman et al., 2004). A number of *Trichoderma* isolates produce a wide variety of fungal cell wall-degrading enzymes such as chitinase, β1-3 glucanase, β1-6 glucanase, cellulases, proteases and used commercially as a source of these proteins. These lytic enzymes that have a high diversity of structural and kinetic properties, some of them were purified, characterized and their encoding genes cloned (Lorito et al., 1988, 1994; Suarez et al., 2004; Montero et al., 2007).

The role of each protein in the enzymatic complex of *Trichoderma* appear to be different and enzymes with different or complementary modes of action appear to be required for maximal antifungal effect on different pathogens (Gajera & Vakharia, 2012; Markovich & Kononova, 2003; Viterbo et al., 2002; Lorito et al., 1994)

Hydrolytic enzymes of *Trichoderma* have many roles in a wide range of different biological systems. Several reports have been given to explain the role of lytic enzymes produce by *Trichoderma* spp. during plant defense. Plating assay is one of the screening methods that have been frequently used. Various plate screening methods for detection of polysaccharides degrading microorganisms have been described in literature. These methods are based on the complex formation between polysaccharides and dyes (congo red, phenol red, remazol brilliant blue and tryphan blue) and observation of visible clear zone around colony (Castro et al., 1995).

The aim of this study was the screening of four *Trichoderma* strains for lytic enzymes production.

## MATERIALS AND METHODS

Four strains of *Trichoderma* from culture collection of RDIPP were used in this study. Stock culture of *Trichoderma* isolates were maintained on potato dextrose agar (PDA) slants at 4°C. Fungal strains were screened for their ability to produce cellulase following the method Congo Red test. Cellulase activities of the fungal strains were determined by using plate screening medium containing (g/L): NaCl (0.5), $K_2HPO_4$ (1), $MgSO_4$x 7 $H_2O$ (0.5), Mn $SO_4$ x $H_2O$ (0.01), $NH_4NO_3$ (0.3), $FeSO_4$ x $7H_2O$ (0.01), CMC (10), agar (15). The pH was adjusted to 6.5 prior to sterilization.

Agar blocks (5mm) from one-week old fungal colony grown on PDA were cut and inoculated in the centre of the basal media plates. The inoculated plates were incubated for 96h at 28°C. For screening step used two replicates per strain. After incubation, plate was stained with 0.1% Congo red dye solution for 15 minutes. Then, the solution was discarded and the cultures were washed with 1M NaCl for 15 minutes. Cellulase production was indicated by the appearance of the halo (hydrolysis zone) surrounding the colonies. This halo was measured and calculation of the enzymatic index (EI) using the next formula (Florencio et al., 2012): EI= diameter of hydrolysis zone /diameter of colony

Also, the strains of *Trichoderma* spp. were screened for producing chitinolytic enzymes by using the method of Agrawal & Kotasthane (2012), based on diameter of purple color zone surrounding the colony of fungi. Medium used for chitinase production had next composition (all amounts are per liter): 4.5g colloidal chitin, 0.3g $MgSO_4$ x 7 $H_2O$, 3g $(NH_4)_2$ $SO_4$, 2g $KH_2PO_4$, 1g citric acid monohydrate, 15g agar, 0.15g bromcresol purple and 200µL tween -80; pH adjusted at 4.7 and autoclaved at 121°C for 15 minutes. Colloidal chitin was prepared from shell crab chitin according to the method described by Robert and Selitrennikoff (1988).

The fresh culture plugs of *Trichoderma* spp. strains to be tested for chitinase activity were inoculated into plates with chitinase detection medium and incubated at 28°C for 3 days and were observed for the colour zone formation. Formation of coloured zone is due used of bromcresol purple in medium, which is a pH indicator dye and transforms the yellow colour of media into purple colour due to increase in pH. Colour intensity and diameter of the purple colour zone were taken as the criteria to determine the chitinase activity exhibited by studied strains.

## RESULTS AND DISCUSSIONS

All cultures of *Trichoderma* were examined qualitatively for production of extracellular enzymes chitinases and cellulases by plate method, through comparing their radial growth. Our results showed that all *Trichoderma* strains studied produced lytic enzymes. The level of production of these enzymes varied depending on strain. The Td49 and Tdal12 strains exhibited higher chitinase activity but Td50 showed low activity (figure 1). The Td49 and Tdal12 strains showed a higher diameter of the purple color zone as compared to Td50 strain. The present finding was also supported by several researchers (Nadarajah et al., 2014; Lunge & Patil, 2012; Agrawal & Kotasthane, 2012). Chitin agar plate has been used earlier for highlighting of chitinolitic microorganisms by some authors. The method used for evaluation of chitinase activity (Agrawal & Kotasthane, 2012) is sensitive easy, semi-quantitative, inexpensive and fast. The method was used also by Kamala & Indira (2011) to evaluate the chitinolitic properties of *Trichoderma* isolates from Manipur against *Fusarium solani*, *Rhizoctonia solani* and *Pythium* pathogens.

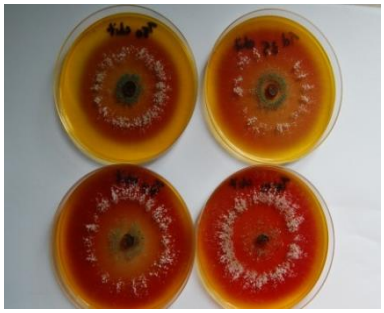

**Figure 1.** Screening of *Trichoderma* strains for chitinase activity on medium with colloidal chitin

All the fungal strains produced zonas of hydrolysis in CMC agar plates within 4 days and results were found in table 1. All the strains studied had highest enzymatic indices with value ranging from 1.18 to 1.5. Most intense cellulolytic activity had Tdal12 and Td49 strains followed by Td50 and Td85 (figure 2).

**Table 1** Enzymatic indices of cellulose for *Trichoderma*

| Strain | Colony diameter(cm) | Hydrolysis zone(cm) | EI |
|---|---|---|---|
| Td49 | 6 | 8 | 1.33 |
| Tdal12 | 5.8 | 8.7 | 1.5 |
| Td85 | 7 | 8 | 1.14 |
| Td50 | 7 | 8.3 | 1.18 |

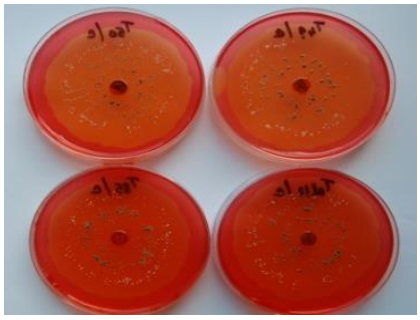

**Figure 2.** Screening of *Trichoderma* strains showing celulase activity on medium with CMC

Yoon et al. (2007) compared the four commonly used chromogenic dyes to determine which dye is favorable for detection of cellulose in diverse fungal species. They found that positive detection of clear zone was most highly and more clearly observed in medium contained red Congo.

## CONCLUSIONS

Based on the results obtained in this study it can be concluded that the medium used is suitable for rapid selection of chitinolitic and cellulolitic strains used sensitive plate assay. All Trichoderma strains studied have produced lytic enzymes such as chitinase and cellulase. Among four strains studied, Td49 and Tal12 were highlighted as chitinolitic activity. Td49 and Tal12 strains had the most intense cellulolytic activity followed by Td85 and Td50 strains

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