RESEARCH PAPER

Asian Journal of Bio Science, Vol. 4 Issue 1 : 29-32 (April to September, 2009)

Screening of *Trichoderma* isolates for chitinolytic activity using glycol chitin plate assay

GANAPATHI BHAT[1], **SUMANGALA BHAT**[2] **AND** M.S. KURUVINASHETTI[2]

[1]Central Food Technological Institute, MYSORE (KARNATAKA) INDIA

[2]Department of Biotechnology, College of Agriculture, University of Agricultural Sciences, DHARWAD(KARNATAKA) INDIA

(Accepted : January, 2009)

Thirty three isolates of *Trichoderma* belonging to different species which were previously screened against *Sclerotium rolfsii*, *Fusarium oxysporum* and *Rhizactonia solani* were used for checking chitinolytic activity using agarose plate containing glycol chitin. Differences were observed among species and isolates of the same species with respect to chitinase production after induction with colloidal chitin and chitin degradation. Among all the isolates, *T. virens* IABT 1010, *T. koningii* IABT1016, *T. polysporum* IABT1018 were found to be more efficient chitinase producers. In all the isolates, chitinase production started within 24 hours of induction, but maximum production reached in 48 hours. Therefore, culture filtrates taken after 48 hours of induction can be used for routine screening of the *Trichoderma* isolates**.**

Key words : *Trichoderma*, Chitinolytic activity, Glycol chitin, Colloidal chitin

INTRODUCTION

Biological control of plant pathogens is an attractive way to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains (Harjono and Widyastuti, 2001). Biological control of plant disease is defined as the involvement and the use of beneficial microorganisms, such as specialized fungi and bacteria, to attack and control plant pathogens and the diseases they cause (Lewis and Papavizas, 1991). Different biological control agents (BCAs) can be used for the control of diseases. These include bacteria, fungi and actinomycetes. The most important BCAs belong to the genus *Trichoderma*. *Trichoderma* controls plant pathogens through several mechanisms including mycoparasitism, antibiosis, production of hydrolytic enzymes such as chitinases and glucanases, inactivation of pathogen's enzyme etc (Chet *et al.*, 1997; Weindling, 1941; Harman *et al.*, 2004). These mechanisms can act synergistically to combat pathogen. However, which mechanism to use at a particular time or pathogen is left to *Trichoderma*. *Trichoderma* adopted best mechanism either alone or in combination through evolution.

There are about 41 species in the genus *Trichoderma*. The species and the isolates within the same species differ in their biocontrol potential (Goes *et al.*, 2002; Umamaheswari and Sankaralingam, 2005; Upendra, 2006). Therefore, isolation and characterization of *Trichoderma* from different geographical locations is likely to provide an array of diverse isolates within desired biocontrol potential against plant diseases. Further, in recent years with the advancement in molecular biology and plant biotechnology, genes encoding chitinases and glucanases from *Trichoderma* are being exploited to develop plant varieties resistant to diseases. In this regard identification of *Trichoderma* isolates which are efficient producer of chitinases are important to use them as a source of chitinase genes. Different methods such as substrate hydrolysis in the medium, substrate degradation and estimation of reducing sugars, glycol chitin plate assay (Luis and Ray, 2004) etc., can be used to detect the chitinolytic activity of any organism. Luis and Ray (2004) used glycol chitin plate assay for chitinase activity in plants. This assay is very efficient, simple and less time consuming. The assay is based on the affinity of calcofluor white M2R for chitin (Maeda and Ishida, 1967) using glycol chitin as a substrate for endochitinase (Pan *et al.*, 1991). Glycol chitin embedded in an agarose gel provides a homogeneous substrate for the reaction to take place (Zou *et al.*, 2002). Glycol chitin serves as substrate for chitinase (Koga and J., 1983) binds to fluorescent brightener 28 by affinity (Maeda and Ishida, 1967). After proper incubation and enzymatic activity, the brightener is bound only to undigested glycol chitin (Trudel and Asselin, 1989). The result is easily visualized as well defined dark area on a fluorescent background when