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A novel gamma radiated mutant of *Trichoderma viride* for biodegeradation of *Pythium ultimum* cell wall

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Abstract

Trichoderma secreted several cellulases and glucanases to lysis the *Pythium* mycelia in mycoparasite process. Enhancement in these enzyme production in *T. viride* by gamma radiation is a useful mutation method, and resulted in increasing antagonistic potential against an important plant pathogen *Pythium ultimum*. In this study, proteomics analysis and cellulase assay were used to indicate the role of endo-glucanase and exoglucanase enzymes in bio-control. The results of cellulase enzyme activity of *T. viride* mutant isolates and its wild type strain after 48 h incubation at 180 rpm and 28 ˚C with different substrate (Avicel, Carboxy methyl cellulose and Bacterial cellulose) are shown the enhancement in the enzyme activity values of the mutant isolates. The specific exo-glucanase enzyme activity in *Tv M21* mutant isolate approximately 2-2.5 times more than its wild type strain and also secreted 3 times more endoglucanase. This superior mutant showed up to 65% growth inhabitation against *P. ultimum* in dual culture test (5 times more than the wild type) and sharper spots belong to endo-glucanase, exoglucanase and β-glucosidase presented in SDS-Page and 2D Electrophoresis of this mutant. Overall, induced mutation by gamma irradiation could be a useful method to access such superior mutants and *TvM21* could be a successful BCAs candidate for plan disease management programs of *Pythium* inoculated soils.

Keywords: Trichoderma viride; γ-irradiation; SDS-Page; 2D Electrophoresis;Cellulase.

Introduction

The genus *Trichoderma* belongs to order Hypocreales is a filamentous fungus widely distributed in the soils of most crops producing areas of Iran. Species of this genus are of great economic importance, as they serve as a source of degrading enzymes (chitinolytic and cellulytic), antibiotics, plant growth promoters and most commercial bio-fungicides (Ozbay and Newman, 2004). *Trichoderma viride* is considered as a potential bio-control agents for many plant pathogens as well as *Pythium ultimum* (Savazzini et al., 2009, Harman et al, 2004). Mechanisms primarily have included direct effects upon target fungi via competition, myco-parasitism, and anti-biosis. Since the production of anti-fungal metabolites (chitinase and cellulase enzymes), appears to be responsible for the ability of *Trichoderma* strains to control the plant pathogenic fungi (such as *Pythium spp.)*, it is hypothesized that superior bio-control activity might be achieved by increasing production levels of these metabolites. Thus, the induced mutation by gamma radiation was used to improve the anti-fungal metabolites in *T. viride* in this study. The optimum irradiation dose was described in previous studies at 250 Gry (Mohamadi et al, 2014).

The cell wall of *Pythium spp.* is characterized by its cellulosic nature, and shown to be composed mainly of carbohydrates (80-90%) plus hexosamines (1-3 %), lipids (3-8 %) and proteins (4%). All species showed a remarkable similarity in the composition of the cell walls, the main difference being in the ratios of the glucans present. Three types of glucans were detected in the cell walls of the *Pythium*: a ß-D-(1,4) - linked glucan (cellulose), alinear ß-D-(1,3) - linked glucan and a linear ß-D-(1,3)-linked glucan with D-glucose substituents attached by ß-(1,6) linkages (Sietsma *et al* 1999). However, even though the *Trichoderma* strains is becoming widely used in commercial agriculture, knowledge of its multiple modes of