ENHANCEMENT OF BIOCONTROL ACTIVITIES OF *TRICHODERMA HARZIANUM* THROUGH PROTOPLAST FUSION

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Introduction

Trichoderma harzianum has been identified as a biocontrol agent against soil-borne pathogens such as *Sclerotium rolfsii* and *Rhizoctonia solani* (Jinantan and Sariah, 1997, 1998). *T. harzianum* is also known to produce a wide array of extracellular enzymes that are involved in the process of antagonism against pathogenic mycelia and sclerotia, and showed highest synergy with other enzymes, or with other biological and chemical control agents (Goldman et al. 1994). It enhances plant growth and is rhizosphere competent. However, this fungus possesses great genetic variability, which makes it less effective and reliable. Therefore it needs to be improved to a more effective strain. Since it lacks sexual stage for genetic manipulation, protoplast fusion is a method of choice. It efficiently induces heterokaryosis (Harman and Hayes, 1993), and allows the recombination in the progeny of different characteristics from two or more parental strains following the removal of cell wall and exposing the protoplast membrane These processes that are less achievable or impossible with intact cells. This study reports on the improvement of biological activities of *T. harzianum* isolates through protoplast fusion.

Materials and Methods

Mycelial cultures from three indigenous isolates of *Trichoderma harzianum* isolated from the rhizospheres of groundnut (IMI 378843), chilli (IMI 378444) and oil palm (IMI 378841) were used for the protoplast isolation and fusion studies. Mycelial cultures at the exponential stage of growth were incubated in the lytic enzyme (Novozyme 234) at a concentration of 7mg /mL. Released protoplasts were confirmed to be viable by determining its germination on Protoplast Regeneration Medium (PRM). The protoplasts were later chemically fused following the modified method of Stasz et al. (1988). The fused protoplasts were plated on PRM and fusants colonies, which grew within 24-48 hr after plating, were isolated and maintained on PDA. The fusants obtained were evaluated for their biological activities based on isozymes, cultural and morphological characteristics, tolerance to sublethal doses of fungicides and as bioprotectants against *S.rolfsii* and *Ganoderma boninense*.

Results and Discussion

Spherical protoplasts were obtained by incubating 2-day-old mycelial cultures in 7mg/mL Novozyme 234. Incubation time of 4h in lytic enzyme solution resulted in an optimum release of viable protoplasts. Pre-treatment of mycelium in 10mM of 2-mercaptoethanol gave no significant difference on protoplast yield among the three isolates of *T. harzianum*. Protoplast fusion was achieved using polyethylene glycol (PEG) with the addition of 0.01M $NaCl_2$ at pH 7.5. The fusion among the respective isolates successfully yielded 12 fusants (A,B,C,D,E,F,G,H,I,J,K,L). The fusants germinated 18h after incubation in liquid PRM solution and regenerated into colonies between 24 and 48 hr after incubation on solid PRM. Of the 12 fusants evaluated for their biological activities, fusants B,C,D,E,F showed non-parental type based on isozymes analysis, but showed similarity in colony growth and microscopic characteristics to their parental isolates. In the dual culture test, fusants D and E showed significantly better antagonism against *S. rolfsii* and *Ganoderma boninense* than their parental isolates. However, in the colony degradation test, the fusants only showed good antagonism against *S. rolfsii* but not against *G. boninense*. Fungal strains that are tolerant to fungicides would form a useful component of an integrated management programme where repeated use of fungicide is important. In this study, the fusants were found to be capable of tolerating sublethal doses of Propiconazole, Penconazole and Quintozene which showed significantly higher tolerance ($P<0.01$) than the target pathogens but weaker than the parental isolates. This may be due to the acquired resistance of the parental isolates to the fungicides over the years in the field whereas the fusants were produced *in vitro* without exposure to fungicides.

Conclusions

A protocol for the isolation and fusion of fungal protoplasts has been developed, which could be used for genetic manipulation of fungi lacking in sexual stage for strain improvement.

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