Genetic Improvement of Trichoderma Fungus for Production of Biodegradation Enzymes

By

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ABSTRACT

Twenty four *Trichoderma* isolates were isolated from agricultural soil samples collected from Fayoum governorates, Egypt. Based on morphological and cultural characteristics, the *Trichoderma* isolates were identified as *Trichoderma harzianum* (12 isolates), *T. lixii* (2 isolates) *T. aureoviride* (8 isolates) and *T. longibrachiatum* (2 isolates). All the twenty four *Trichoderma* isolates showed positive test for cellulase as revealed by the formation of a yellow zone on the screening medium containing Congo red as indicator. The percentage inhibitory effect of all the twenty four *Trichoderma* isolates against growth of *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* was calculated and ranged from 52.9% to 67.6% against of *R. solani,* 35% to 65.6.% against *F. solani* and ranged from 44.4% to 63.9% against of *M. phaseolina. T. harzianum* (FUGT3), *T. lixii* (FUGT6), *T. Longibrachiatum* (FUGT16) were showed the greatest antagonistic effect to the *R. solani* (67.6%), *T. harzianum* (FUGT3) showed the greatest antagonistic effect to the *F. solani* (65.6%) whereas *T. lixii* (FUGT6), *T. Longibrachiatum* (FUGT16) and *T. aureoviride* (FUGT18) showed greatest antagonistic against *M. phaseolina* (63.9%). Cellulase producing from *Trichoderm* isolates was determined spectrophotometry and four *Trichoderma s*trains (FUGT3, FUGT6, FUGT16 and FUGT18) showed highest CMCase activities (0.32, 0.34, 0.36 and 0.42 IU/ml), respectively. Molecular phylogenetic analysis was performed based on nucleotide sequences. The results indicate that the isolat4es. FUGT3, FUGT6, FUGT16 and FUGT18 are closely related to *T harzianum, T. lixii, T. Longibrachiatum* and *T. aureoviride* with accession number OL953189 and OL953190*,* OL953191 and OL953192, respectively. The four selected strains were subjected to mutation using UV radiation and chemical mutagens (Ethyl Methano Sulphate and sodium azide). The results showed that following, 167 mutants were obtained, 48 mutants induced by UV, 55 mutants induced by EMS and 64 mutants induced by sodium azide. These mutants were tested for their cellulase productivity compared to their wild type and the results showed that, the wild strains exhibited the highest cellulase activity 0.42 IU/ml on carboxy methyl cellulose (1.0%)**.** Upon mutation by UV exposure the *Trichoderma* mutants produced cellulase 0.921 IU/ml whereas EMS and Sodium azide treated mutant showed 1 IU/ml cellulase activities**.** The results showed that all mutants induced from the wild type *Trichoderma* (FUGT3, FUGT6, FUGT16 and

FUGT18) were significantly better than the wild type when tested against *R. solani*, with mutant FUGT3S60 and FUGT3S75 scoring the highest growth inhibition at 77.8% and 79.4%, respectively, whereas the FUGT3 wild type showed 64.9%. Also the inhibition % of mutants induced from FUGT16 was high significantly than the wild type, with mutant FUHT16E100 scoring 82.2% whereas the inhibition % of wild type FUGT16 (67.6%). The ISSR-PCR approach has shown to be useful for assessing the genetic diversity of *Trichoderma* isolates and their corresponding mutants. More also, the mutants from UV and chemicals were selected and ISSR analysis of genomic DNA was performed to detect genetic diversity of these mutants with the wild type by using 6 primers. The existence of the (cbh1) gene, encoding for the exo-cellobiohydrolas, in the genome of *Trichoderma* and their mutants was confirmed by PCR using specific primers for *Trichoderma* cbh1 genes. A DNA fragment of 200 bp was amplified from the genome of the strains.
Keywords: *Trichoderma*, Cellulase activity, Cbh1 gene, ISSR, Antagonistic, mutation, UV, EMS, sodium azide