Identification and characterization of biocontrol genes and their functions from *Trichoderma* isolates against soil borne fungal plant pathogens

By

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APPROVAL SHEET

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Summary

The present study was carried out in Genetics Department, Faculty of Agriculture, Fayoum University, Egypt, during the period from ٢٠٠٧ to 20١3. Eighteen isolates of *Trichoderma* spp. previously isolated from the rhizospheres of different plants (Faba bean, Bean, Cucumber, Cowpea and Wheat) growing in Fayoum Governorate, and two reference strains (*T. harzianum* and *T. koningii*) obtained from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

Therefore, the objectives of this study were

(1) To identify *Trichoderma* isolates using morphological and molecular characters.

(2) To evaluate the potential of isolates as biological control agents of *Rhizoctoni solani*.

(3) To isolate the chitinase and cellulase producing *Trichoderma* isolates.

(4) Isolation of their chitinase, cellulase genes and detect the sequence of these genes.

The obtained results were summarized as the follows:

Eighteen isolates of *Trichoderma* spp. previously isolated from the rhizospheres of different plants growing in Fayoum Governorate. Morphological and molecular characterization of antagonistic ability of *Trichoderma* species were conducted. On the basis of morphological and cultural characteristics, the *Trichoderma* isolates were identified as *T. harzianum* (10 isolates), *T. koningii* (8 isolates), and *T. viride* (2 isolates).

All fungal *Trichoderma* isolates inhibited the growth of *Rhizoctonia solani* which caused the root rot disease on Faba bean and varied in their antagonistic effect.

The isolate FUE_3 showed the highest inhibition (57.77%) of *Rhizoctonia solani* growth. On the other hand, FUE20 showed the lowest inhibition (25.33%). The remaining isolates showed intermediate values percentage of inhibition growth of *R. solani*.

Results indicated that the maximum amount of chitinase 0.018 mg/ml excreted by I18 isolate. The minimum amount of chitinase 0.0047 mg/ml excreted

by FUE10 and I12 isolates. Excretion of chitinase by the rest of *Trichoderma* isolates ranged from 0.017 to 0.005 mg/ml.

Results indicated that the maximum amount of cellulase 0.075 mg/ml excreted by FUE5 isolate. The minimum amount of cellulase 0.006 mg/ml excreted by FUE8 and FUE13 isolates. Excretion of cellulase by the rest of *Trichoderma* isolates ranged from 0.035 to 0.007 mg/ml.

Under greenhouse, soil infestation singly with *Trichoderma* greatly reduced the percentages of pre- and post-emergence damping off caused by *R. solani*. After 15 day, FUE6 and I18 isolates were the most effective. *Trichoderma koningii* I18 was the most effective one followed by *Trichoderma koningii* FUE6 after 30 days from sowing. *Trichoderma harzianum* FUE15 was the most effective one for root rot disease.

All six isolates produced PCR products of similar size for the rDNA region. A single product of approximately 560 to 600 bp was obtained from all the PCR amplifications with primers ITS1 and ITS4 for 6 biocontrol isolates of *Trichoderma* spp. The highest homology (100%) was found between isolates FUE5, FUE6 and FUE3, FUE5. While the less homology (92%) was found between isolate FUE15 and all isolates except isolate FUE9 (93%).

Based on the results obtained all the six isolates can be grouped into two main clusters. One cluster represents *T. harzianum* (FUE15) and other *T. konigii* (FUE3, FUE5, FUE6, FUE9 and I18). The GenBank accession number for the ITS region of rDNA sequence of *Trichoderma* is KC200070, KC200071, KC200073, KC200074 and KC200075 for *Trichoderma koningii* FUE3, *T. koningii* FUE5, *T. koningii* FUE6, *T. koningii* FUE9 and *T. harzianum* FUE15, respectivelly.

A specific band about 1039 bp was amplified from *Trichoderma* chromosomal DNA. It was observed that specific band appeared in all tested *Trichoderma* isolates. The primers used were useful to identify the presence of the chitinase gene in *Trichoderma* isolates.

Upon complete analysis, the full length chitinase gene isolated from *Trichoderma* isolates had 1039 bp, encoding 344 amino acid. Multiple alignment of the deduced amino acid sequence with related fungal proteins was performed with the CLUSTAL W 2.1 program when *Trichoderma* isolates chit36 was compared with the previously reported chitinase sequences of ABG56440-*T. asperellum*, ABC48784-*T. asperellum*, AAL01372-*T. harzianum* and ABO14715-

T. atroviride. The results in table 8 showed that, using pairwise alignment, the homology found between the ABO14715-*T. atroviride chi36* and chitinase protein from FUE3 *T. konigii* and FUE9 *T. konigii* was 100%. The homology between the ABG56440-*T. asperellum* and the ABC48784-*T. asperellum* equal the homology between the FUE3 *T. koningii* and the FUE9 *T. koningii* (100%). The less homology between the I18 *T. koningii chi36* and chitinase protein from ABC48784-*T. asperellum* and AAL01372-*T. harzianum* was 96%.

The results showed that, using pairwise alignment, the homology found between the *T. harzianum* FUE15 *cbh-1* and celullase protein from AFD01232-*T. harzianum* was 97.1%. The homology between the *T. harzianum* FUE15 and the ADH04808-*T. harzianum* (94.2%). The less homology between the AFD01232- *T. harzianum* and ADH04808-*T. harzianum* was 94.06%.

The length cellulase gene isolated from *Trichoderma* isolates had 204 bp. The dendrogram of *Trichoderma* isolates for celullase gene showed that, the dendrogram was divided into two clusters. The first cluster included *T. harzianum* FUE15 and AFD01232-*T. harzianum*, while the second cluster included ADH04808-*T. harzianum*.