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Molecular improved *Trichoderma* showed better biocontrol activity in lab and greenhouse

M Kowsari[1], S Mahmoodian[2], M Motallebi[2] and **M R Zamani[2]**
[1]Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Iran
[2]National Institute of Genetic Engineering and Biotechnology (NIGEB), Iran

Biological control of plant diseases is one of the effective strategies to reduce harmful effects of pesticides on human health and environment. *Trichoderma* is one of the most successful biological control agents. The biocontrol activity of *Trichoderma* strains usually correlates with the secretion of cell wall degrading enzymes such as chitinases. Chitinase Chit42 is believed to play an important role in the biocontrol activity of *Trichoderma* strains as a biocontrol agent against phytopathogenic fungi. Chit42 lacks a chitin-binding domain (ChBD) which is involved in its binding activity to insoluble chitin. In this study, *T. harzianum* was co-transformed with the plasmid p3SR2 and the pLMRS3 derivatives (pLMRS3-chit42 and pLMRS3-chit42-ChBD). Plasmid p3SR2 carries the amdS gene from as Aspergillus nidulans, which codes for acetamidase as a selectable marker. The chimeric chitinase was constructed by the fusion of a Chit18-10 ChBD from T. atroviride to Chit42. The prediction of the ChBD glycosylation site by NetOGlyc 3.1 server showed four glycosylation sites in the Ser-rich linker which separated the catalytic domain from the binding domain. The glycosylation of linker prevented the chimeric enzyme from proteolysis which occurs mainly in this region. The ChBD was added to the N-terminal of chit42 employing SOEing PCR. To test the expression of chit42 and chit42-ChBD in the selected transformants (*Trichoderma chit42*-ChBD 3, 6, 7, 11, 13 & 15) quantitative RT-PCR was performed using real time PCR The improved chitinase containing a ChBD displayed a 1.7 fold higher specific activity than chit42. This increase suggests that the ChBD provides a strong binding capacity to insoluble chitin. Moreover, Chit42-ChBD transformants showed higher antifungal activity towards important phytopathogenic fungal species (*R. solani, F. graminearum, F. oxysporum, S. sclerotiorum, V. dahlia, A. brassicales* and *B. cinerea*) after that biocontrol assay was done in dual culture method. The minimum and maximum values of mean Inhibition by Chit42-ChBD transformants against the seven phytopathogenic fungi were 31.6% and 88.6% respectively, which were significantly different compared with wild type (10%-49.5%). The presence and stability of the chimeric protein was confirmed by molecular technique. The biocontrol activity and colonization of improved isolates were evaluated on bean plant and its pathogen (*Rhizoctonia solani*) in greenhouse. The results showed that transformant *Trichoderma chit42*-ChBD 3 with 82/53 percent inhibition compared to control, was the best biocontrol in *in vitro*. According to the results of greenhouse tests, plants that were treated with *Trichoderma chit42*-ChBD 7, 3 and 15 in all stages of measurement (two leaf, mid-term growth, early reproductive stage and harvest stage) showed symptoms less than 30%. Root surface colonization and fungal hyphae penetration was confirmed by molecular studies using primers PF1/R3xbaI. In conclusion, our data demonstrate that enzyme engineering can produce a chitinase with an improved binding capacity, which will lead to higher enzyme and antifungal activities; thus the transformants generated in this study might result in better biocontrol agents in the field.

Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO) Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO)

Biography

She has completed her PhD (Mlecular engineering) from National Institute of Genetic Engineering and Biotechnology (NIGEB) department of plant biotechnology. She is member of several academic society. She has published papers in reputed journals. She is a member of academic staff of Agricultural Biotechnology Research Institute of Iran (ABRII). Molecular Improvement of *Trichoderma* is the most important section of her research.

kowsari@abrii.ac.ir

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