Jittima Aeungkittikul 2008: Cloning of Endochitinase Gene from *Trichoderma harzianum* into Yeast (*Pichia pastoris*) and Efficacy Evaluation of Recombinant Yeast Culture Filtrate for The Control of Yard Long Bean Damping-Off . Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Wanwilai Intanoo, Ph.D. 69 pages.

The chitinase enzyme by *Trichoderma* species is of interest in relation to their use in biocontrol and as a source of mycolytic enzyme. The *chi42* gene encoded a 42 kDa endochitinase was cloned into the *E. coli* cloning vector but a result of the expression in *E. coli* expression vector, pQE80L, was undesirable because of an insoluble protein. Therefore, in this study, production of a soluble secreted molecule from the recombinant yeast expression system for expressing a soluble protein into the medium was investigated and evaluated for its application in the biological control of yard long bean damping-off. Endochitinase (*chi 42* gene) encoding cDNA was isolated from total RNA of *T. harzianum* strain CB-Pin-01 by RT-PCR. The *chi42* cDNA coded for an endochitinase of 1275 nucleotide sequences. The *chi42* gene from *T. harzianum* was cloned in pCR®8/GW/TOPO cloning vector, prior to subclone into *Stu*I site in the *Pichia pastoris* expression vector, pPIC9. From 72 transformed yeast clones, the integration of chitinase gene (1275 bp in nucleotide sequence) in the genome of recombinant yeast was successfully confirmed by PCR (23 positive clones) and Southern blot hybridization in 6 recombinant yeast clones. From phylogenetic tree, sequence comparison showed that *chi 42* gene from this study and other chitinases available in GenBank had more than 90% similarity in nucleotide sequences. Although the analysis of recombinant protein using SDS-PAGE can not examine the expressed 42 kDa protein in the culture medium of recombinant yeast, endochitinase activity from the supernatant of recombinant yeast culture was clearly detected, comparing to the control treatment (non-transformed *P. pastoris*). From an evaluation of disease-control efficacy, yard long bean seed soked in culture filtrate from the recombinant yeast showed higher efficiency by 62.5% for controlling yard long bean damping-off caused by *Rhizoctonia solani*, comparing to the control treatment with pathogen only. The use of culture filtrate secreted from the recombinant yeast, however, gave less efficient inhibition to the fungal disease when compared to the application of *T. harzianum* suspension and Carboxin. The efficient production of recombinant chitinase from the biocontrol agent was achieved when the disease incident between a recombinant yeast and non-transformed yeast was exhibited, 35% and 50%, respectively.

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