DATE: Day 13 Month May Year 2019

SUMMARY of

2018 RESEARCH RESULTS REPORT

For International Collaborative Research with IPR, Osaka University

Research Title		**Chemical synthesis of the defensin peptide derived from Nicotiana megalosiphon to evaluate the fungal resistance in soybean transgenic lines**
Applicant	**Name**	**Hilda Elisa Garay Pérez**
	Affiliation	**Center for Genetic Engineering and Biotechnology (CIGB)**
	Present Title	**Senior Researcher. Head of Synthetic Peptides Group**
Research Collaborator (Host PI)		**Hironobu Hojo (Professor)**

Summary

Plant diseases caused by fungi and oomycetes have an important impact for crop production in Cuba and worldwide. Every year huge amounts of fungicides for control are applied, with the effect thereof on the environment and human health. Find alternatives with biotechnological approaches for disease control is the biggest challenge for researchers related with this topic. Plant defensins are small, cysteine-rich peptides, which inhibit the growth of a wide range of microorganisms and are part of the defense mechanisms of plants against diseases. The application of the plant defensin as alternative to reduce crop losses due to the attack of pathogen constitutes an advantage with regard to the application of chemical fungicides. First: plant defensin are derived from seeds, roots and tubers, for what they constitute nature substances that are not toxic to the host plant and neither to people that consume the products from these plants. Second: as other protein, the defensin quickly degrades like native substances not leaving any residual after their effectiveness expires. This defensin was isolated from a plant and develops a strong anti- microbe activity in vitro and ex vitro against important bacteria, fungus and oomycetes pathogens. Herewith, the constitutive expression of this defensin in transgenic plants increased the resistance to many plant diseases with high economic impact. In the present work, the chemical synthesis of defensin linear peptide (45 aa) from Nicotiana megalosiphon was carried using the solid phase peptide approach. Besides, it was studied five folding condition to identify the optimal condition that favors the presence of one major isomer (Condition I: Redox buffer (GSH/GSSG). Condition II: DMSO at 10%. Condition III: Redox buffer (GSH/GSSG) and organic solvent (Glycerol 10%). Condition IV: DMSO at 20%. Condition V: DMSO at 20% and denaturant reagent (GuHCL)). In the five conditions studied, there is not presence of linear peptide after 24 h and various isomers of the folding peptide were formed. The use of redox buffer (GSH)/GSSG) does not promote efficiently the formation of one main isomer with adequate percentage in the isomers mixture. The addition of glycerol as organic solvent in the redox buffer condition delays the folding reaction. The use of DMSO as oxidative reagent was the more effective protocol for folding the defensin peptide and the addition of GuHCL as denaturant reagent does not enhance the folding behavior. The ESI-MS analysis demonstrated that the folded peptide have the four disulfide bonds. This result will be included in a research paper and it will be presented in the 5th International Symposium on Synthetic Peptide as Human and Veterinary Pharmaceutical Products “Synthetic Peptides 2019” (June 28th – July 1st, 2019), Cuba.

***Deadline: May 17, 2019**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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