

<b>Title</b>	CLONING, EXPRESSION AND CHARACTERIZATION OF ALPHA- AMYLASE INHIBITOR FROM KDML105 THAI RICE ( <u>ORYZA SATIVA</u> L.)
<b>Author</b>	Natthawut Poomsila
<b>Advisor</b>	Associate Professor Sukkid Yasothornsrikul, Ph.D.
<b>Co-Advisor</b>	Sittiruk Roytrakul, Ph.D.
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### ABSTRACT

Rice (*Oryza sativa* L.) is one of the most important crops and a staple food for almost half of world's population. However, stored rice seeds are vulnerable to insect pests that feed on rice grain and cause loss of its value and quality. There are alpha-amylase inhibitors in plants, which can inhibit insect alpha-amylases, leading to undergrowth of insect pests. Nevertheless, there is no report on the nucleotide sequences and expression of alpha-amylase inhibitor genes from Thai rice, especially cultivar Kaw Dok Mali 105 (KDML 105). Moreover, specificity and selectivity between the insect alpha-amylases and inhibitors from plants have not yet been elucidated. Therefore, the aim of this study is to clone the full coding sequence of alpha-amylase inhibitor genes from KDML105 rice in order to further study their expression and inhibition activity. In this study, we have cloned a cDNA from young leaves of KDML105 rice. The clone consists of 603 nucleotides, encoding an alpha-amylase inhibitor. Its nucleotide and deduced amino acid sequences are identical to those of japonica rice alpha-amylase/subtilisin inhibitor. The 603 nucleotides of gene were expressed in BL21 *E.coli*. The expressed protein was peptide confirmed using LC-MS/MS and was partially purified using ion exchange chromatography. Inhibition activities was assayed using 2-Chloro-4-nitrophenyl- $\alpha$ -D-maltotrioxide (CNPG3), resulted in functional alpha-amylase inhibitor which decreased 68.82% and 83.52% of alpha-amylase activities of human saliva and *Bacillus subtilis*, respectively. In

contrast, it did not inhibit alpha-amylase recombinant activity from *Tribolium castaneum*. However, these results revealed the sequence of KDML105 alpha-amylase inhibitor differs from the indica Homjan alpha-amylase inhibitor, another clone from our group, indicating a complex family of proteinaceous inhibitors in rice that could lead to complex interactions of alpha-amylases. Moreover, the KDML105 alpha-amylase inhibitor can be reduced amylase activity from human saliva and microorganism, suggesting possible application of the KDML105 alpha-amylase inhibitor in microbial control and medicine.