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VORARATT CHAMPATTANACHAI : CLONING AND SEQUENCE ANALYSIS OF β -GLUCOSIDASE/ β -FUCOSIDASE ENZYME FROM THAI ROSEWOOD. THESIS ADVISOR : JISNUSON SVASTI, Ph.D., RUTAIWAN TOHTONG, Ph.D., JAMES R. KETUDAT-CAIRNS, Ph.D., 144 p. ISBN 974-589-366-8

A β -glucosidase/ β -fucosidase extracted from the dry seed of Thai Rosewood has been purified and characterized in our laboratory. The germinated seed of Thai Rosewood was used to extract RNA for cloning the cDNA encoding this enzyme. Three peptides from the amino-terminal and lysine-C and trypsin digested peptides sequenced in our laboratory were used to design degenerate oligonucleotide primers. These primers were used to amplify a β -glucosidase transcript fragment using total RNA extracted from the germinated seeds by reverse transcription and polymerase chain reaction (RT-PCR). A specific PCR product of approximately 250 bp encoding a β -glucosidase fragment was produced using these degenerate primers, and this PCR-product was cloned into pGEM-T plasmid, transformed into *E. coli* DH5 α , and finally sequenced using an automated DNA sequencing. The cloned β -glucosidase fragment is composed of a 224 base pair DNA sequence which could be translated into a 74 amino acid sequence. DNA sequence of this fragment was used to synthesize specific primers to amplify the full length cDNA of this enzyme using rapid amplification of cDNA ends (3'RACE and 5'RACE) with the RNA extracted from geminated seeds in the same manner. The resulting full length β -glucosidase cDNA sequence is composed of 1,955 bases of DNA sequence which can be translated into a 547 amino acid sequence, including a signal peptide of about 23 residues, in addition to the 524 residue mature protein. Moreover, the amino acid sequence of cloned β -glucosidase also contains 14 peptide sequences determined by protein sequencing of the amino-terminal purified and peptides isolated from the protein using lysine-C and trypsin digestion, and its amino acid composition is very similar to the amino acid composition of the purified enzyme.

Multiple sequence alignment analysis indicates that the amino acid sequence of Thai Rosewood β -glucosidase has the highest sequence identity with the β -glucosidase from sweet cherry (*Prunasin serotina*) (56.7%) and the cyanogenic β -glucosidase (linamarase) from white clover (*Trifolium repens*) (56.3%). Moreover, the result suggest that this enzyme should be classified into glycosyl hyrolase family 1. Such comparisons also suggest that the conserved catalytic nucleophile residue of active site in the enzyme is Glu₄₁₉ (Glu₃₉₆ in the mature protein), while the conserved catalytic proton donor residue is Glu₂₀₅ (Glu₁₈₂ in the mature protein).