

# Proceedings

The 1<sup>st</sup> International Conference on  
Rice for the Future



31 August - 3 September 2004  
Kasetsart University, Bangkok

ISBN 974-537-534-9

## Organized by

- Kasetsart University(KU)
- National Center for Genetic Engineering and Biotechnology (BIOTEC)
- Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative

## Recombinant Protein Expression, and Functional Characterization of a Putative Cell Wall-Bound $\beta$ -Glucosidase from Rice

Busarakum Pomthong<sup>1</sup>, Rodjana Opassiri<sup>1\*</sup>, Tasanee Onkoksoong<sup>1</sup>, Takashi Akiyama<sup>2</sup>, James R. Ketudat-Cairns<sup>1</sup>

<sup>1</sup>Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand;

<sup>2</sup>Department of Low Temperature Science, National Agricultural Research Center for the Hokkaido Region, Sapporo 062-8555, Japan; \*opassiri@ccs.sut.ac.th

$\beta$ -glucosidases constitute a major group among glycoside hydrolases and they play key roles in a variety of biological process (e.g., growth and development, chemical defense, cellulolysis etc.). In plants, these enzymes are likely to have many as yet undetermined functions. Analysis of rice gene sequences in the Monsanto, Rice Genome Sequencing Project, Beijing Genomic Institute and Torrey Mesa Research Institute databases showed at least 47 genes homologous to  $\beta$ -glucosidase (Glycosyl Hydrolase Family 1). The 445-1 contig containing the deduced amino acid sequence which appeared to be very similar to the previously purified and characterized cell wall-bound  $\beta$ -glucosidase (Akiyama et al., 1998) was selected to study the biological functions. The 445-1 full-length cDNA was cloned by RT-PCR from rice seedlings, and sequenced completely. The cDNA encoding mature 445-1 was cloned into pENTR4 Gateway plasmid and subcloned into various expression vectors by using LR clonase system (Invitrogen). The 445-1 protein was expressed as recombinant protein in *Escherichia coli*. This enzyme hydrolyzed p-nitrophenyl  $\beta$ -D-glucoside and other  $\beta$ -O-linked glycosides, and also oligosaccharides. These results indicated one possible function of the enzyme might be further hydrolysis of oligosaccharides released from cell wall  $\beta$ -glucans during seed germination.