MOLECULAR CLONING, CHARACTERIZATION AND EXPRESSION ANALYSIS OF TWO CELLULASE EGXS FROM GOLDEN APPLE SNAIL (POMACEA CANALICULATA)

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Cellulase is an enzyme that catalyzes the degradation of celluloses. The full-length cDNA of two cellulases were isolated and characterized from stomach tissue of Golden Apple Snail (Pomacea canaliculata) by Rapid Amplification of cDNA End (RACE)-PCR. Analysis of nucleotide sequences revealed two isoforms of EGX-C101 and EGX-C103. The open reading frames (ORF) of cellulase cDNA were 1188 and 1191 nucleotides, encoding 396 and 395 amino acid residues, respectively. The calculated molecular mass of the mature protein is approximately 44 kDa. Comparative and bioinformatic analysis revealed that EGX-C101 and EGX-C103 have high homology with glycosyl hydrolase family 10 (GHF10) and had an overall similarity of 98% and 82% to those of Ampullaria crossean EGX. A phylogenetic tree showed a clear differentiation of each species and also indicated that P. canaliculata EGX and A. crossean EGX are closely related phylogenetically. The genomic organization of cellulase EGX-C101 and EGX-C103 genes was determined. The EGX-C101 and EGX-C103 genes spanned over 4937 and 4512 bp, respectively. Both genes contained nine exons and eight introns where all boundaries conformed the GT/AG rule. Expression analysis by RT-PCR revealed that neither gene was expressed in eggs. EGX-C101 was also expressed in 1 and 7-day-old juvenile snail whereas EGX-C103 was expressed only in a 1-day-old juvenile snail.

Keywords: Pomacea canaliculata, cellulase, cloning, golden apple snail

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Introduction:
Cellulases belong to the large group of glycosyl hydrolases, of which there are several families based on amino acid sequences similarities. Golden apple snails are herbivores with an ability to consume large amounts of several kinds of vegetation such as rice seedlings, taro, duckweed, algae, azolla and other succulent leafy plants. Serious destructive damage of rice crops by golden apple snails has been reported since 1996 and it is now regarded as being the most harmful pest to rice seedlings in Thailand. The research presented in this paper investigates the molecular information of potential composition of Cellulolytic enzymes in golden apple snails. The full-length cDNA, the genomic organization and mRNA expression of cellulase gene at various stages of development were also determined.

Method:

Cloning of full length cellulase EGXs
Total RNA was extracted from stomach tissues of P. canaliculata using the TRizol Reagent. First stranded cDNAs were synthesized using Impront-II Reverse Transcription System. The PCR products were cloned and sequenced. To obtain the full length EGX cDNA, the 3′-rapid amplification of the cDNA end (RACE) was performed using gene-specific primer corresponding to the cDNA sequence obtained from the internal amplification. The PCR products were cloned and sequenced.

Characterization of genomic structure of cellulase EGX genes
Genomic DNA was extracted from the foot tissue of each snail using Phenol-chloroform-proteinase
K method. PCR were performed with the Expand Long Template PCR system using primers located in the 5′ and 3′ UTR. PCR was carried out as the following temperature profiles. 94 °C for 3 min followed by 5 cycles of 94 °C for 1 min, 55 °C for 1.30 min and 68 °C for 4 min, followed by 25 cycles of 94 °C for 1 min, 65 °C for 1.30 min and 72 °C for 4 min. The final extension was carried out at 72 °C for 10 min. The PCR product were cloned and sequenced.

Expression analysis of cellulase EGX
RT-PCR was carried out to investigate the expression profile of EGX in different developmental stages including the egg, 1- and 7-day-old juvenile snails. PCR product was digested with Rsa I and analyzed in 1.8% agarose gel electrophoresis.

Results and Discussion:
Cloning and characterization of cellulase EGX cDNAs:
The full-length cDNA of two isoform cellulases (EGX-CI01 and EGX-CI03) were obtained using RACE-PCR. The EGX-CI01 cDNA was 1300 bp containing a 1188 bp open reading frame (ORF) encoding 395 amino acids. The EGX-CI03 cDNA was 1277 bp containing a 1191 bp open reading frame (ORF) encoding 396 amino acids. Comparative and bioinformatic analysis revealed that EGX-CI01 and EGX-CI03 have extensive identity with glycosyl hydrolase family 10 (GHF10) and had an overall similarity of 98% and 82% to those of Ampullaria crossean EGX.

Genomic structure of cellulase EGX genes:
The genomic organization of EGX-CI01 and EGX-CI03 genes were determined by PCR. The EGX-CI01 and EGX-CI03 genes spanned over 4937 and 4512 bp, respectively. Comparison between the genomic sequences and the cDNA sequences revealed that both genes composed of 9 exons and 8 introns (Fig. 1). The sequences of all the exon-intron boundaries conformed to the typical eukaryotic splice sites, including an invariant GT at the intron 5’ boundary and an invariant AG at its 3’ boundary. The result verified the endogenous origin of the EGX genes.

Expression analysis:
Expression analysis of EGX-CI01 and EGX-CI03 genes was investigated by RT-PCR analysis. The result showed that none of EGX transcripts was detected at the egg stage. EGX-CI01 was also expressed in 1 and 7-day-old juvenile snail whereas EGX-CI03 was expressed only in a 1-day-old juvenile snail. The result showed that two EGX transcripts were developmentally expressed.

Figure 1 Schematic diagrams of P. canaliculata EGX-CI01 (A) and EGX-CI03 (B) cDNAs and genes. Non–coding regions are represented by solid bars. Introns (with numbers) are gray-shaded.

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References: