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Isolation and Characterization of cDNA Clones Encoding Thionin from the Fast Growing Shoots of *Bambusa edulis* Murno

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Objectives

To identity the function of thionin, we sequenced the thionin clones, compared the sequences with the thionin genes from other species, and performed a digital northern analysis using the microarray databases constructed with rice genes.

Materials and Methods

Sequencing and sequence analysis

Sequencing reactions were performed using a MJ Research PTC-225 Thermal Cycler with ABI PRISM BigDye Terminator Cycle Sequencing Kits and AmpliTaq DNA polymerase (Applied Biosystems). The samples were subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). Sequence analysis was done using CLC Main Workbench program (CLC Bio) and ClustalX program (Larkin et al., 2007). Visualization of amino acid sequence comparison of bamboo thionin genes was performed using BOXSHADE 3.21 program (http://www.ch.embnet.org/software/BOX_form.html).

Phylogenetic analysis

Thionin family (PF00321) sequences were obtained from PFAM database (http://pfam.sanger.ac.uk/). Sequence alignment was performed using ClustalX program and and trees was constructed using neighbor joining method and the tree was viewed using TreeView program (Page, 1996).

Digital northern analysis

Digital northern analysis was performed using the Genevestigator website, https://www.genevestigator.com/gv/index.jsp (Hruz et al., 2008). Options were as follows, Oryza sativa as an organism, 305 microarray were used, two thionin genes (Os.6645, Os.12973) from rice Unigene database were used as probes.

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Results

Thionin genes from ESTs of *B. edulis*

From the EST database search, 54 thionin clones were found and the sequences were classified into 8 groups and named as *Bethi1* through *Bethi8*. The numbers of clones of each gene ranged from 1 to 16. We sequenced the 8 genes to identify the full length sequences.

Amino acid sequence analysis

The number of amino acid was 135 for 6 thionin genes and 136 for 1 gene and 154 for the other gene. All the putative amino acid sequences have 12 conserved cysteine residues. *BeThi3*, *BeThi4*, *BeThi5*, and *BeThi6* encode identical amino acid sequences even though the nucleotide sequences are different.

Phylogenetic analysis of thionin from *B. edulis*

The phylogenetic analysis of bamboo thionin protein sequences with typical thionin proteins of plants showed that the thionin of fast growing shoots were separated to three groups and the closest protein were thionins of rice and *Crambe abyssinica*. Viscotoxin of *Viscum album* is grouped together with *B. edulis* thionins. Viscotoxin is a plant defensive protein against microbes (Urech et al., 2007; Urech et al., 1995). Bamboo thionins may have defensive function to protect the soft region of fast growing shoots and it could be a potential candidate for selective treatment for cancer as the viscotoxin (Tabiasco et al., 2002). Crambin is a close group to the bamboo thionin, BeThi7. Therefore BeThi7 might be responsible for the sweet taste of the bamboo shoots (Hendrickson and Teeter, 1981; Teeter et al., 1981).

Gene number comparison between plant species

The large number of thionin genes from growing region of bamboo shoot is comparable with the number of genes of other species. From the NCBI (http://www.ncbi.nlm.nih.gov/) UniGene database, 24, 9, 8, 8, 8, 5, and 3 thionin genes were found for *Arabidopsis thaliana*, *Raphanus sativus*, *Raphanus raphanistrum*, *Triticum aestivum*, *Zea mays*, *Hordeum vulgare*, and *Capsicum annuum*, respectively. We could not find any thionin genes for bamboo from the existing database.

Digital northern of thionin genes from rice

To predict the thionin gene expression from bamboo, digital northern analysis was performed from rice, the closest model plant to bamboo. We performed the analysis using the Genevestigator web site (https://www.genevestigator.com/gv/index.jsp). We found the expression pattern of two rice thionin genes. All rice thionins, including Japonica and Indica types, were grouped together with bamboo thionins found from this study. The thionin genes of rice were highly expressed at the germinating, seedling, and shoot growing stages. The genes was expressed very low level at the other stages. To find the exact expression pattern, real-time RT-PCR analysis using gene specific primers should be done for the fast growing bamboo shoots.