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Cloning and Gene Manipulation of a cDNA Encoding A Cellulase Produced by the Mulberry Longicorn Beetle, *Apriona germari*

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Objectives

We have cloned and sequenced the cDNA coding for a cellulase from the mulberry longicorn beetle, *Apriona germari,* with the polymerase chain reaction. And then we have constructed the recombinant plasmid vector for *Bombyx mori* transfomation experiment.

Materials and Methods

The mulberry longicorn beetle, *Apriona germari* was obtained from the Dong-A University (Busan, Korea). The *piggyBac*-derived vector was obtained from the National Institute of Agrobiological Sciences (Tsukuba, Ibaraki, Japan). Genomic DNA extraction and gene manipulation was carried out according to Maniatis et al, (1982). PCR amplification was done by using a "ReadyMix Taq PCR Reaction Mix" (Sigma). According to the sequences, two primers, Ag-F (GAA TCC ATG AAG GTA TTG TTG) and Ag-R (GCG GCC GCT TAT GAA TAA TTG), were designed. Cloned sequences were labeled with the Taq Dye Deoxy Terminator Cycle sequencing Kit. Labeled samples were sequenced wih a 373A DNA Sequencer.

Results and Discussion

From the mulberry longicorn beetle, *Apriona germari,* cDNA libray, a clone homologous to other cellulase gene was isolated using the polymersae chain reaction. As a result of PCR using genomic DNA, 700-bp fragment was specifically amplified and this fragment was used sequencing (Fig. 1). In the nucleotide sequence, the 700-bp open reading frame coding for a polypeptide of 239 amino acid residues was detected which showed high homology with other cellulase gene of the mulberry longicorn beetle(Fig. 2).

On the other hand, we constructed a recombinant plasmid, containing cellulase gene of the mulberry longicorn beetle, for germline transformation of the silkworm *Bombyx mori* L. using a *piggyBac* transposon-derived vector (Fig. 3). The transformation vector constructs consist of the *piggyBac* inverted terminal repeats flaking a fusion of the *B. mori* cytoplasmic actin promotor, the green fluorecent protein (GFP) and cellulase gene.

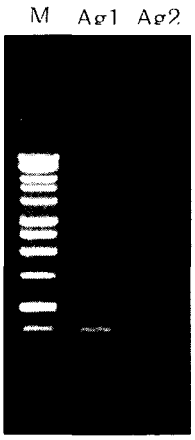

Fig. 1. Result of PCR by the genomic DNA of the mulberry longicorn beetle, *Apriona germari*. Amplification of the 700-bp DNA fragment of the cellulase gene(lane 1, 1-kb DNA ladder; lane 2 and 3, cellulase gene)

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     *
  1  ATGAAGGTATTGTTGGCAGTCGTCGCTGTGCTCTGCACATTCGAAGCATCGTTGTCCCAG    60
  1  M  K  V  L  L  A  V  V  A  V  L  C  T  F  E  A  S  L  S  Q
 61  GACTACCACGTGACACCACTGGTAGGCGGTGTGAGTGGTACCGGCAAAACCACCCGCTAC   120
 21  D  Y  H  V  T  P  L  V  G  G  V  S  G  T  G  K  T  T  R  Y
121  TGGGACTGTTGCAAACCATCATGCTCTTGGAAGGCTAACTTGAAGAACAAATCTGGTAAA   180
 41  W  D  C  C  K  P  S  C  S  W  K  A  N  L  K  N  K  S  G  K
181  CCCGTAGAAGCTTGCGCGGCTGATGGAAAGACAGTAGTGAAGGAGAGTACAAAATCGGCT   240
 61  P  V  E  A  C  A  A  D  G  K  T  V  V  K  E  S  T  K  S  A
241  TGTGAAGAAGGCGCTGGTGCCTACATGTGTTCCGATCAGCAACCCAAAGTGGTTAACTCC   300
 81  C  E  E  G  A  G  A  Y  M  C  S  D  Q  Q  P  K  V  V  N  S
301  ACATTCGCTCTGGGATACGTCGCGGCTTCCTTCACTGGTGGAATTGATGTCAACATGTGT   360
101  T  F  A  L  G  Y  V  A  A  S  F  T  G  G  I  D  V  N  M  C
361  TGTGCTTGTTTGCGTCTCAAGTTCCAGGGCGACCTTTCGGGCAAACAGATGATTGTCCAA   420
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Fig. 2. Nucleotide sequence of cellulase gene of the mulberry longicorn beetle, *Apriona germari*, and the deduced amino acid sequences. An asterisks show the position of the start and termination codon, respectively.

Fig. 3. Structure of the *piggyBac*-derived vector, containing the cellulase coding sequence. A3, *B. mori* cytoplasmic actin gene promoter; GFP+Ag, GFP fused to the cellulase coding sequence; SV40, polyA termination codon; ITR, inserted terminal repeats of *piggyBac*.

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