

Two Homologous Parasitism-specific Proteins Encoded in *Cotesia plutellae* Bracovirus (CpBV) and Their Expression Profile in *Plutella xylostella*

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A wasp, *Cotesia plutellae*, parasitizes diamondback moth, *Plutella xylostella*, and alter host physiology for wasp survival and development. To identify parasitism-specific factors is required for understanding host-parasitoid relationship. This study found ≈5 kDa protein specific to the plasma of parasitized *P. xylostella*. Degenerate primers were designed after the N-terminal amino acid sequence of the parasitism-specific protein (PSP15) and used to clone the corresponding gene from the parasitized *P. xylostella* by a nested reverse transcriptase-polymerase chain reaction (RT-PCR). The cDNA of PSP15 α was cloned as 607 bp, of which open reading frame (ORF) encoded 158 amino acid residues with a signal peptide (21 N-terminal residues). During cloning PSP15 α , another high homologous gene was cloned as PSP15 β , which exhibited the same size of open reading frame and identical N and C terminal sequences with PSP15 α .

Both genes were located in segment "M" of *C. plutellae* bracovirus genome. Real time quantitative RT-PCR (qRT-PCR) indicated late expression of both PSP15 genes after parasitization, where a common major peak was at 7-8 days after parasitization. Both PSP15 α and β were expressed in bacterial expression system and purified as ≈20 kDa recombinant proteins, which were used for raising polyclonal antibody. An immunoassay supports the qRT-PCR data of the PSP15 expression. The recombinant PSP15 α and β proteins could enter hemocytes of non-parasitized *P. xylostella*.

Amino acid sequences of PSP15 α and PSP15 β were homologous to malaria circumsporozoite protein, known to inhibit host protein synthesis. They also shared sequence homologies with eukaryotic translation initiation factors, eIF4F and eIF5. We put PSP15 α ORF into *Autographa californica* multi-nucleocapsid nuclear polyhedrovirus (AcMNPV), and a resulting recombinant exhibited more potent pathogenicity than control AcMNPV.

Key words: Parasitism, *Cotesia plutellae*, *Plutella xylostella*, Polydnavirus, parasitism-specific proteins, Circumsporozoite protein, Eukaryotic translate initiation factors