Screening of binding proteins that interact with Chinese sacbrood virus VP3 capsid protein in *Apis cerana* larvae cDNA library by the yeast two-hybrid method

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**ABSTRACT**

Chinese sacbrood virus (CSBV) causes larval death and apiary collapse of *Apis cerana*. VP3 is a capsid protein of CSBV but its function is poorly understood. To determine the function of VP3 and screen for novel binding proteins that interact with VP3, we conducted yeast two-hybrid screening, glutathione S-transferase pull-down, and co-immunoprecipitation assays. Galectin (GAL) is a protein involved in immune regulation and host-pathogen interactions. The yeast two-hybrid screen implicated GAL as a major VP3-binding candidate. The assays showed that the VP3 interacted with GAL. Identification of these cellular targets and clarifying their contributions to the host-pathogen interaction may be useful for the development of novel therapeutic and prevention strategies against CSBV infection.

1. Introduction

Chinese sacbrood virus (CSBV) is an important pathogen of *Apis cerana* species and causes larval death before pupation, eventually leading to loss of the entire colony (Li et al., 2006). CSBV was first described in Guangdong, China in 1972, and re-emerged in Liaoning, China in 2008 (Ma et al., 2010). Chinese sacbrood disease occurs most frequently in the spring, when the colony is growing most rapidly and large numbers of susceptible larvae and young adults are present (Bailey, 1969). Larvae infected by CSBV failed to pupate, and ec dysial fluid rich in virus accumulated beneath their unshed skin to form a sac. The infected larvae change in color from pearly white to pale yellow, and shortly after death they dry out, forming a dark brown gondola-shaped scale (Bailey, 1975).

CSBV is a picorna-like virus in the genus *Iflaviridae*, which is an icosahedral, non-enveloped viral particle 26–30 nm in diameter. It has a single positive-stranded RNA genome approximately 8.8 kb in size with a large open reading frame (Chen et al., 2006; Choe et al., 2012). The main open reading frame encodes a polypeptide that is comprised of four structural proteins (VP1, VP2, VP3, and VP4). VP1, VP2, and VP3 form the CSBV capsid protein, but little is known about VP4 (Ghosh et al., 1999; Ma et al., 2011; Yu et al., 2016). The VP3 gene is approximately 1005 base pairs (bp) in length, and the approximate molecular weight of the encoded protein is 31.5 kDa (Bailey et al., 1982). In addition, the VP3 protease site (GAAQQ1078LTAS) conforms to the classic pattern for viral 3C proteases that cut after either glutamine (Q) or glutamic acid (Ma et al., 2011). Structural proteins play an important role in viral infection, replication, and pathogenicity (Zorina and Zorin, 2013; Fernández-Sainz et al., 2014; Hussmann et al., 2014). However, the specific host cell interaction partner proteins and function of CSBV capsid proteins are unknown.

The yeast two-hybrid (Y2H) method (Fields and Song, 1989) is a powerful tool for identifying binary protein–protein interactions by exploiting the modular nature of the yeast Gal4 transcription factor. The galectin 4 (GAL4) protein is composed of two main fragments: a DNA-binding domain and a DNA activation domain. A bait protein is expressed as a fusion to the GAL4 DNA-binding domain when the GAL4 gene is activated. In the Y2H system, the DNA-binding domain and activation domain of GAL4 are fused to two proteins of interest. If these two proteins physically interact, an active GAL4 transcription factor is generated, driving the expression of reporter genes under the control of the GAL promoter (Wong et al., 2017). Y2H screens have been used to study viral interactions with host cell factors (Rajagopala et al., 2012; Wang et al., 2017; Ward et al., 2017; Sun et al., 2018).

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To clarify the functions of VP3, we identified host cellular proteins that interact with this VP3 capsid protein using the Y2H system and *Apis cerana* larvae cDNA libraries. The resulting clone was sequenced and a similarity search was performed using the BLAST and BLASTx tools in public sequence databases. The results indicated that VP3 interacts with the GAL host protein. Glutathione S-transferase (GST) pull-down and co-immunoprecipitation (Co-IP) assays showed that VP3 interacts with GAL. The results suggest that GAL ameliorates CSBV pathogenesis and host antiviral defense.

## 2. Materials and methods

### 2.1. Plasmid, Escherichia coli, cDNA library, virus, and reagents

Plasmids pET28a, pCDNA3.1, pPIC9K-VP3-His, pCDNA3.1-VP3-His, and pGEX-6P-1, purified CSBV, 2–3-day-old *A. cerana* larvae, and yeast cDNA libraries of *A. cerana* larvae were constructed in our laboratory. *Escherichia coli* DH5a and BL21 were purchased from TransGen Biotech (Beijing, China). *Saccharomyces cerevisiae* strains Y2HGold and Y187 and other YPs for the Y2H system kit, including yeast selective culture medium,YPD medium, isopropyl β-D-thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-a-GAL), were purchased from Clontech (Mountain View, CA, USA). T4 DNA ligase and restriction enzymes were purchased from Takara (Shiga, Japan). Dulbecco’s modified Eagle’s medium and fetal bovine serum were purchased from Corning Corporation (Corning, NY, USA). GST Sefinose™ Resin was purchased from Sangon Biotech (Shanghai, China). The Pierce BAC protein assay kit was used (Rockford, IL, USA). Antibodies and anti-Flag M2 Magnetic Beads were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Bait plasmid and plasmid construction

Total RNA was extracted from purified CSBV and 2–3-day-old *A. cerana* larvae using Trizol Reagent following the manufacturer’s protocol (Invitrogen). The RNA was reverse-transcribed into cDNA using a first-strand cDNA synthesis kit. Four pairs of primers for pGBK7-VP3 (bait plasmid), pGADT7-GAL, pGEX-GAL, and pCDNA3.1-GAL-Flag were designed. Restriction enzyme sites were inserted based on the CSBV mRNA sequence (GenBank accession No. HM223761.1) and *A. cerana* galectin gene (GenBank accession No. XM 392379.5) (Table 1). The PCR cycling conditions were an initial denaturation at 94 °C for 2 min followed by 30 cycles at 94 °C for 45 s, 58 °C for 45 s (pGBK7-VP3), or 55 °C for 60 s (pGADT7-GAL, pGEX-GAL, and pCDNA3.1-GAL-Flag), and 72 °C for 60 s, and a final extension step for 10 min at 72 °C. The PCR products were purified, double-digested, extracted, and inserted into the vector by the restriction enzyme sites. Subsequently, the plasmid was transformed into *E. coli DH5a* and verified by endo-nuclease cleavage and sequencing (Sangon Biotech).

### 2.3. Auto-activation and toxicity detection of bait protein expression plasmid pGBK7-VP3

To test the auto-activation of the bait protein, the correctly constructed plasmid pGBK7-VP3 and empty pGBK7 plasmids were transformed into the Y2HGold yeast strain. Transformants were grown on SD/-Trp, SD/-Trp/X-a-GAL (40 μg/mL X-a-GAL) and SD/-Trp/X-a-GAL/AbA (40 μg/mL X-a-GAL and 125 ng/mL Aureobasidin A) agar plates for 3–5 days. Toxicity of the bait protein expression plasmid pGBK7-VP3 was detected based on the growth of the colonies on the plates.

### 2.4. *A. cerana* larvae cDNA library screening

All yeast strains and reagents for the Y2H assays were purchased from Clontech Co. To identify host cellular proteins that interact with VP3, we screened this protein against a honeybee cDNA library. Briefly, Y187 cells, which have harbored the *A. cerana* larvae cDNA library cloned into the pGADT7 vector, were mated with Y2HGold cells transformed with pGBK7-VP3 for 24 h at 30 °C. The mated cells were spread on SD/-Leu/-Trp/X-a-GAL/AbA plates (DDO/X/A). Blue colonies were patched out onto higher stringency SD/-Ade/-His/-Leu/-Trp/X-a-GAL/AbA (DDO/X/A) plates and grown for another 4–6 days.

### 2.5. Confirmation of positive interactions and rescue of prey plasmid

Yeast colonies grown on the DDO/X/A agar could potentially interact with bait and prey proteins. They were subjected to colony PCR and rescue of the plasmid for subsequent analysis. Briefly, potential positive colonies detected by PCR after oscillation culture were sequenced by Shanghai Biotechnology Bioengineering Co. Ltd. (Shanghai, China). The sequence was searched against the NCBI/GenBank databases. The potential positive prey plasmid (pADT7-GAL) from the initial screen was transformed into the Y187 strain, followed by mating with the Y2HGold yeast strain containing the pGBK7-VP3 bait plasmid or empty pGBK7 vector. The mated cultures were spread on DDO/X/A agar. Y2HGold transformed with the pGBK7-53 or pGBK7 vector were used to mate with Y187 cells containing pGADT7-T and were used as a control.

### 2.6. Expression and purification of GST and GAL fusion proteins from *E. coli*

The plasmids pGEX-GAL were transformed into *E. coli* BL21(DE3). A single bacterial colony was selected from the transformants and used to inoculate 5 mL of LB medium and incubated at 37 °C for 10 h. The cultures (1000 μL) were inoculated into 50 mL LB (100 μg/mL Amp) and cultured at 37 °C until the absorbance at 600 nm reached 0.6. Protein expression was induced by adding 0.5 mM IPTG at 28 °C for 5 h. The cell pellets were collected to check the expression of GAL by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). GST-fusion proteins were further purified using GST Sefinose™ resin.

### Table 1

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>Restriction enzyme sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGBK7-VP3</td>
<td>VP3 F</td>
<td>5’-GGGATTTCGACATCTGGTGCTAAAGAGATG-3’</td>
<td>EcoR I</td>
</tr>
<tr>
<td>pGBK7-VP3</td>
<td>VP3 R</td>
<td>5’-GGTACGAGTTGGTCTCGGATAGAT-3’</td>
<td>Pst I</td>
</tr>
<tr>
<td>pGBK7-VP3</td>
<td>GAL F</td>
<td>5’-GGATCTAGTAAGATTGGTCTCGGATAGAT-3’</td>
<td>BamH I</td>
</tr>
<tr>
<td>pGBK7-VP3</td>
<td>GAL R</td>
<td>5’-GGCGGATGTGTTGCTCGGATAGAT-3’</td>
<td>Xho I</td>
</tr>
<tr>
<td>pGADT7-GAL</td>
<td>GAL F</td>
<td>5’-GGAAATTTACATGATCAAGGATGACGAGATATATATA-3’</td>
<td>Xho I</td>
</tr>
<tr>
<td>pGADT7-GAL</td>
<td>GAL R</td>
<td>5’-GGGATGTTAATCTGCTCGGATAGAT-3’</td>
<td>Xho I</td>
</tr>
</tbody>
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