Open reading frame Bm21 of Bombyx mori nucleopolyhedrovirus is not essential for virus replication in vitro, but its deletion extends the median survival time of infected larvae

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In this report, the open reading frame 21 (Bm21) of Bombyx mori nucleopolyhedrovirus (BmNPV), one of the unique genes of group I NPVs, was characterized. Bm21 is predicted to encode a protein of 55.8 kDa and was found to contain imperfectly conserved leucine-rich repeats. 3′ Rapid amplification of cDNA ends (3′ RACE) showed that the transcript of Bm21 was first detected from 6 h post-infection and that it also encompassed the complete Bm20. 5′ RACE revealed three transcription initiation sites, one of which mapped to the baculovirus early transcription motifs CGTGC and CAGT. Transient-expression and superinfection assays indicated that BM21 localized in the nucleus of infected BmN cells. To study the function of BM21, a Bm21-null virus was constructed using bacmid technology. Viral one-step growth curve analyses showed that the Bm21-null virus had similar budded virus production kinetics to those of the parental virus. Bioassay analyses showed that the median lethal concentration (LC50) of the Bm21-null virus was similar to that of the control virus; however, the median survival time (ST90) of the knockout virus was significantly longer than the control virus. These results indicate that Bm21 is not essential for virus replication in vitro, but that deletion of the gene delays the killing of the infected larvae.

INTRODUCTION

The lepidopteran nucleopolyhedroviruses (NPVs) can be divided into two groups, I and II, based on the phylogenetic studies of baculoviruses (Zanotto et al., 1993; Bulach et al., 1999; Herniou et al., 2001). Autographa california multicapsid NPV (AcMNPV) and Bombyx mori NPV (BmNPV) are examples of group I NPVs, whilst Lymantria dispar NPV, Spodoptera exigua MNPV and Helicoverpa armigera single-nucleocapsid NPV belong to group II. Genomic comparison has revealed that there are unique group I genes that are conserved only in group I NPVs and do not exist in other baculoviruses. Currently, there are 11 unique group I genes: ac1 (protein tyrosine phosphatase 1 gene, ptp1), ac16 (ADV-e26), ac30, ac42 (global transactivator gene, gta), ac72, ac73, ac114, ac124, ac128 (gp64), ac132 and ac151 (ie2) (E. A. Herniou, personal communication; Ikeda et al., 2006). Many of the unique group I genes have been characterized to some extent. For example, three genes encode structural proteins: PTP1 is present in both the budded virus (BV) and occlusion-derived virus (ODV) (Li & Miller, 1995), and can induce enhanced locomotory activity in infected larvae (Kamita et al., 2005); BV/ODV-E26 is a structural protein of the envelope of BV and ODV (Beniya et al., 1998), and is a palmitoylated multifunctional structural protein associated with DNA binding (Burks et al., 2007) and co-localizes with IE1 (Imai et al., 2004); and gp64 encodes a viral envelope fusion protein that is responsible for viral attachment, fusion and egress (Blissard & Wenz, 1992; Hefferton et al., 1999; Oomens & Blissard, 1999). Two of the unique group I genes are involved in viral DNA replication and gene expression: ie2 encodes a protein that functions as an activator of viral gene expression (Carson et al., 1988; Yoo & Guarino, 1994a, b; Shippam-Brett et al., 2001; Mainz et al., 2002) and is involved in viral DNA replication (Kool et al., 1994; Lu & Miller, 1995) and arrest of the cell cycle (Prikhod'ko & Miller, 1998), whilst gta has been suggested to function in opening up viral chromatin and to aid in the binding of virus-specific transcription factors to viral DNA based on their sequence homology to a family of DNA-stimulated
ATPases (Lapointe et al., 2000). So far, the functions of the six unique genes ac30, ac72, ac73, ac114, ac124 and ac132 remain to be characterized.

Open reading frame 21 of BmNPV (Bm21) is a homologue of ac30, one of the six uncharacterized unique genes of group I NPVs. Bioinformatics analyses have shown that Bm21 and its homologues are tryptophan-repeat genes, which contain multiple, imperfectly conserved, leucine-rich repeats (LRRs) (Dall et al., 2001). LRR elements can mediate protein–protein interactions and LRR proteins have been reported to participate in many biologically important processes, such as neural development (Mutai et al., 2000), cell polarization (Bilder & Perrimon, 2000), regulation of gene expression (Linhoff et al., 2001), apoptosis signalling (Inohara et al., 1999), plant disease resistance (Jones & Jones, 1997) and bacterial virulence (Reisner & Straley, 1992; Marino et al., 1999; Evdokimov et al., 2001).

In this paper, the transcription of Bm21 was analysed and the function of the Bm21 was studied by constructing a Bm21-null recombinant virus. The in vitro and in vivo infectivity of the recombinant virus was compared with the parental virus. The subcellular location of Bm21 was also studied by constructing a fusion protein with enhanced green fluorescent protein (EGFP). These studies provided insight into the function of this unique group I gene.

METHODS

Insect cell line, larvae and virus. The BmN cell line was cultured at 28 °C in TC-100 insect medium (JRH Biosciences) supplemented with 10% fetal bovine serum (Gibco-BRL) using standard techniques (O’Reilly et al., 1992). Larvae of the silkworm B. mori (Dazao) were reared in groups at 27 ± 1 °C on an artificial diet (Choudary et al., 1995) and third- or fifth-instar larvae were used in the experiments. BmNPV bacmids BmBacJS13 and BmBacJS13-ph derived from BmNPV Shaanxi strain were constructed previously in our laboratory (Huang et al., 2007).

Sequence analysis of Bm21. Primers 21F with a BamHI site (5'-GGGAGGCATCCATGTGTTACATATTGTAGTAGGA-3', introduced enzyme digestion site underlined) and 21R with an XhoI site (5'-GGGGCTCGAGACTTAAATTATAATTTGTATCGTGACGAC-3') were designed to amplify Bm21 from BmBacJS13. The PCR product was cloned into a pGEM-T Easy vector (Promega) and three positive clones were sequenced. The amino acid sequences predicted from the sequences of Bm21 were analysed against a database (www.expasy.org).

Transcriptional analysis of Bm21. BmN cells were infected with vBmBacJS13-ph at an m.o.i. of 5 TCID50 units per cell. Total RNAs were extracted with Trizol (Invitrogen) following the manufacturer’s instructions from infected cells at 0, 4, 6, 8, 12, 24, 36, 48 and 72 h post-infection (p.i.). 3' Rapid amplification of cDNA ends (3'RACE) was performed using avian myeloblastosis virus reverse transcriptase (Takara) and an oligo(dt)15 three-site adaptor primer (5'-CTGATCTAGAGTACCCGGATCCTTTTTTTTTTTTTTTTTTTTTTT-3') using 1 µg purified total RNA as template to synthesize the first cDNA according to the manufacturer’s instructions. The PCR was carried out using the cDNA as template. The primers were a three-site adaptor primer (5'-CTGATCTAGAGTACCCGGATCCTCCG-3') and a Bm21-specific forward primer (5'-GACCGCCCAATTACTGGTG-3'). The PCR products were analysed by agarose gel electrophoresis and the product of the 48 h p.i. sample was purified and cloned into pGEM-T Easy. Five positive clones were sequenced to examine the 3' end of the Bm21 transcript.

The 5’ ends of Bm21 transcripts were determined using total RNA collected at 48 h p.i. According to the manufacturer’s recommendations (SMART RACE cDNA Amplification kit user manual; Clontech), the first-strand cDNA for 5'RACE was synthesized using superscript II reverse transcriptase with the Bm21-specific primer GSP1 (5'-CGATTGTGGGAGATTGTACGGCC-3') and the SMART A primer (5'-AAGCACTGGTATCAACGAGTAGTATTCCCAGG-3'). After cDNAs were generated, the RACE-PCR was conducted with the SMART A primer and primer GSP2 (5'-GACCGGGTGTAGGCTTTGGTGG-3'). The PCR products were gel purified and cloned into pGEM-T Easy for sequencing.

Localization of Bm21 in the BmN cells. A plasmid containing the Bm21-egfp fusion gene was constructed to study the localization of Bm21 in BmN cells. A fragment containing the egfp gene was digested from pEGFP-N1 (Clontech) with EcoRI and NotI, and subcloned into pIZ/V5-His (Invitrogen) to generate pIZ/V5-egfp. The BmN cell sequence without the stop codon (TAA) was amplified by PCR from BmBacJS13 using a forward (5'-CGGTTACATGTGTTATACATTTACATTGTAACC-3'), KpnI site underlined) and reverse (5'-GGGATCCAAATTATTCTCATTTGTATCGGGACAT-3'; HindIII site underlined) primer and inserted into pIZ/V5-egfp to generate pIZ/V5-Bm21-egfp. Plasmid pIZ/V5-Bm21-egfp was transfected into BmN cells with Lipofectin (Invitrogen) following the manufacturer’s protocol. Plasmid pIZ/V5-egfp was used as a negative control.

Superinfection of BmN cells was conducted with vBmBacJS13-ph (m.o.i. of 10 TCID50 units per cell) at 12 h post-transfection (p.t.). At 60 h p.t., the cells were stained with Hoechst dye and observed under a confocal laser-scanning microscope (Leica).

Construction of Bm21-knockout bacmid BmBacJS13A21 and polyhedral-repaired bacmid BmBacJS13A21-ph. The Bm21 deletion bacmid was constructed using the chloramphenicol resistance (CmR) to replace Bm21. Specifically, the primer pair 5'-TTATTATTGTGTTACATATTGTGTAAGCAGTACGTTCCAAGGCGGTACACTAAAGCACAATTACGGCTTFA-3' and 5'-ATTAGGTTAATTATTTCTCATTGTATACCGGACACCTTGTTGG-3' (ntcodons to homologous to the Bm21 region are underlined) was designed to amplify a linear fragment containing CmR and with 57 bp upstream and 62 bp downstream flanking sequences of Bm21. Using these primers, a 1.1 kb PCR product containing the CmR gene was amplified from pBeloBac11 (Invitrogen). The linear PCR product was gel-purified and transformed into Escherichia coli BW25113 containing BmBacJS13 DNA together with the helper plasmid pKD46 providing the phages λ Red recombination (Hou et al., 2002). Bm21-deletion bacmids were obtained by homologous recombination in E. coli and screened using kanamycin and chloramphenicol resistance. Recombinant bacmids were identified by PCR and restriction enzyme digestion, and the correct bacmid was designated BmBacJS13Δ21.

The BmNPV polyhedrin gene (ph) was introduced into BmBacJS13Δ21 by transposition in E. coli using pFastDual-ph (Huang et al., 2007) according to the Bac-to-Bac system manual. The resulting bacmid was named BmBacJS13A21-ph.

Transfection and infection of BmN cells to obtain recombinant viruses. To obtain recombinant viruses, 5 x 10^3 BmN cells were transfected with 1 µg bacmid DNA (BmBacJS13-ph or BmBacJS13A21-ph) using Lipofectin (Invitrogen) as described by the manufacturer. BVs of the recombinant viruses were harvested at 4 days p.t. and used to infect BmN cells. The supernatants were
collected at 96 h p.i. and viral titres were determined by end-point dilution assay (EPDA).

**Comparison of BV one-step growth curves of vBmBacJS13-ph and vBmBacJS13Δ21-ph.** BmN cells (2 × 10⁶) were infected with vBmBacJS13-ph or vBmBacJS13Δ21-ph at an m.o.i. of 5 TCID₅₀ units per cell. At the appropriate time points (0, 6, 12, 24, 36, 48, 72 and 96 h p.i.), supernatants were collected and the titres of the BVs determined using EPDA. Polyhedral inclusion bodies (PIBs) were used as a marker of infection during the assay. Each viral infection was carried out in triplicate and the BV titres at different times p.i. were log-transformed and statistically analysed using the repeated measure method in a general linear model (SPSS Inc.).

**Bioassays.** PIBs of recombinant viruses (vBmBacJS13-ph or vBmBacJS13Δ21-ph) were purified from third-instar-infected *B. mori* larvae. The median lethal concentration (LC₅₀) of the viruses was determined by feeding third-instar larvae (n=50) an artificial diet containing different concentrations of PIBs. The mortality of the infected larvae was observed daily and the LC₅₀ values determined by probit analysis (SPSS Inc.). The LC₅₀ values of the two viruses were compared further using a two-sided z-test (Snedecor, 1989).

The median survival time (ST₅₀) of the viruses was determined with third-instar larvae using a food-contamination method. PIBs (4 μl of 5 × 10⁶ PIBs ml⁻¹) of the viruses were applied to a small plug of artificial diet in individual containers, and only larvae that completely ingested the contaminated diet within 5 h were reared further (n>40). Time 0 was defined as the point at which the larvae were placed on the contaminated diet and mortality was recorded at intervals of 4 h. ST₅₀ values of the two viruses were calculated using the Kaplan–Meier estimator and further compared using the log-rank test (Kalbfleisch & Ross, 1980).

**RESULTS**

**Sequence analysis of Bm21**

As the BmNPV bacmids (BmBacJS13 or BmBacJS13-ph) we used were derived from the Shaanxi strain of BmNPV of which the entire genome has not been sequenced (Huang et al., 2007), Bm21 was amplified from BmBacJS13 by PCR for sequencing. Three PCR clones were sent for sequencing. All of the sequences were identical. The coding region of Bm21 was sequenced, and their results were identical. The coding region of Bm21 strain sequenced to the wild-type BmNPV Shaanxi strain.

A BLAST search revealed that all group I NPVs sequenced to date contain homologues of BM21 (Fig. 1). BM21 and its homologues all contained LRRs with 23 aa repeats represented by X-W-X2-L-X5-L-X4-L-X6-L (Dall et al., 2001). The LRR copies of BM21 were imperfect, with variation in both framework sequence and length (Fig. 1).

**Transcriptional mapping of the 3’ and 5’ ends of Bm21 transcripts**

The timing of Bm21 transcription and the 3’ end of the Bm21 transcripts were determined using 3’RACE. As shown in Fig. 2(a), an RT-PCR product of 1.0 kb was first detected at 6 h p.i. and this band continued to be detected until 72 h p.i. Five clones containing the fragment of the 48 h p.i. RT-PCR product were sequenced. All of the sequences were identical and it was shown that the transcription product contained not only Bm21, but also the coding sequence of Bm20 (Fig. 2b). There were two poly(A) signals (AATAAA) located downstream of the stop codon of Bm20 (Fig. 2b). The 3’RACE results suggested that the second AATAAA, located 61 nt downstream of the Bm20 stop codon, was used as the poly(A) signal for the Bm21 and Bm20 co-transcript (Fig. 2b).

The 5’ ends of the Bm21 transcripts were determined by 5’RACE analysis using total RNA collected at 48 h p.i. The results revealed three different transcription initiation sites located 116, 89 and 81 nt upstream of the Bm21 translation initiation codon ATG (Fig. 2b). The second initiation site (−89 nt) was positioned at an overlapping C residue (shown in bold) of a CGTG motif and a CATG motif (Fig. 2b). Both CGTG and CATG are baculovirus early transcription motifs. No conventional early or late motifs were identified around the other two initiation sites positioned at G (−116 nt) and C (−81 nt) residues.

**BM21 localizes in the nucleus of infected cells**

To study the localization of BM21 in insect cells, pIZ/V5-Bm21-egfp in which the egfp gene was fused in frame to the C terminus of Bm21 was used to transfect BmN cells. The intracellular localization of BM21 was examined by confocal laser-scanning microscopy. As shown in Fig. 3, the fusion proteins were located exclusively in the nucleus of transfected cells (Fig. 3b). When BmN cells were superinfected with vBmBacJS13-ph, the localization of BM21 remained in the nucleus of infected cells (Fig. 3d). However, in the negative controls, EGFP showed homogeneous fluorescence in the cytoplasm and nucleus when expressed alone (Fig. 3a) or when superinfected with vBmBacJS13-ph (Fig. 3c). Therefore, BM21 is likely to localize in the nucleus of infected cells.

**Construction of recombinant bacmids BmBacJS13Δ21 and BmBacJS13Δ21-ph**

To analyse the role of the Bm21 gene during virus infection in BmN cells and *B. mori* larvae, recombinant bacmids BmBacJS13Δ21 and BmBacJS13Δ21-ph were constructed (Fig. 4a). The recombinant bacmids were examined by PCR (data not shown) and restriction endonuclease analyses. Fig. 4(b) shows an XbaI digestion profile of the recombinant bacmids. As predicted, the E fragment (11.6 kb) of BmBacJS13 was replaced by a smaller E' fragment (11.2 kb) in BmBacJS13Δ21 and BmBacJS13Δ21-ph due to the Cmᵣ
Fig. 1. Amino acid sequence alignment of BM21 homologues. Sequence alignment was carried out using CLUSTAL_X 1.83 and adjusted using GeneDoc software. The imperfect LRR motifs (X-W-X2-L-X5-L-X4-L-X6-L; Dall et al., 2001) are indicated by lines above the sequences. The sequences used were: AcMNPV orf30 (GenBank accession no. L22858), Plutella xylostella NPV (PlxyNPV) orf29 (DQ457003), Rachiphusia ou MNPV (RoMNPV) orf27 (AY145471), Maruca vitrata NPV (MaviNPV) orf21 (EF125867), Epiphyas postvittana NPV (EppoNPV) orf30 (AY043265), Antheraea pernyi NPV (AnpeNPV) orf115 (DQ486030), Choristoneura fumiferana defective NPV (CIDFNPV) orf33 (AY327402), Anticarsia gemmatalis NPV (AgMNPV) orf37 (DQ813662), Hyphantria cunea NPV (HycuNPV) orf117 (AP009046), Choristoneura fumiferana MNPV (CfMNPV) orf33 (AF512031) and Orgyia pseudotsugata MNPV (OpMNPV) orf38 (U75930).
substitution. When the ph gene was introduced into the attTn7 target site of BmBacJS13 D21 or BaBacJS13, H fragments (4.9 kb) were substituted by 7.0 kb H9 fragments in BmBacJS13 D21- and BmBacJS13-(Fig. 4b). The results indicated that all recombinant bacmids were correctly constructed.

Virus replication in BmN cells and one-step growth curves of BV production

After transfection of recombinant bacmid BmBacJS13A21 or BmBacJS13A21-ph into BmN cells, the supernatants could successfully infect BmN cells (data not shown), indicating that Bm21 is not essential for virus replication. BmN cells infected with vBmBacJS13A21-ph showed similar cytopathic effects to vBmBacJS13-ph, for example, at 48 h p.i., detachment of cells and the presence of PIBs could be observed within infected cells. To analyse BV yields of the recombinant viruses, one-step BV growth curves were determined by EPDA. The results (Fig. 5) showed that vBmBacJS13A21-ph and vBmBacJS13-ph had similar dynamics of BV production, as the titre of vBmBacJS13A21-ph was similar to parental virus at similar stages of infection ($F=2.431$, d.f.$=1,4$, $P=0.194$).

LC50 and ST50 assays of recombinant viruses

To determine the LC50 values of vBmBacJS13A21-ph and vBmBacJS13-ph, third-instar B. mori larvae were infected orally with selected doses of PIBs and monitored for mortality. The results are shown in Table 1. Statistic analysis indicated no significant difference in the LC50 values between the two viruses ($z=0.286$, $P>0.05$). ST50 values of vBmBacJS13A21-ph and vBmBacJS13-ph were determined by orally feeding third-instar B. mori using a food-contamination method. The ST50 of vBmBacJS13-ph was $116\pm1.2$ h, whilst that of vBmBacJS13A21-ph was $132\pm1.4$ h (Table 1).
Fig. 4. Construction and identification of recombinant bacmids. (a) Schematic representative genomes of BmBacJS13 and the recombinant bacmids. The location and orientation genes are indicated by arrows. (b) Identification of recombinant bacmid DNAs by XbaI digestion. Lanes: M₁, λ DNA digested by HindIII; 1, BmBacJS13; 2, BmBacJS13Δ21; 3, BmBacJS13-ph; 4, BmBacJS13Δ21-ph; M₂, λ DNA digested by BamHI, EcoRI and HindIII.

DISCUSSION

*Bm21* is one of the uncharacterized unique genes of group I NPVs. In this paper, the transcription of *Bm21* and the subcellular location and function of the protein were studied.

Transcription analysis showed that *Bm21* was transcribed from 6 h p.i. and accumulated to high levels from 12 to 48 h p.i. (Fig. 2a), indicating a combination of early/late promoters. 5′RACE revealed three transcription initiation sites located 116, 89 and 81 nt upstream of the ATG start codon (Fig. 2b). The second transcription initiation site mapped to the baculovirus early transcription motifs CGTG and CAGT, and is probably responsible for the early transcription of *Bm21*. Although the baculovirus late transcription motif TAAG was not identified in the upstream region of *Bm21*, the late transcription of *Bm21* may be initiated from the other two initiation sites located 116 and 81 nt upstream of ATG. Alternatively, it is also possible that some of the transcripts were initiated from the early stages of infection and were kept steady until the late stages of infection. Further experiments need to be performed to identify the transcription pattern of each initiation motif.

Transient and superinfection analyses showed that BM21 was located exclusively in the nucleus, indicating that BM21 may conduct its function in the nucleus during a natural infection. Although no classical nucleus location signal (NLS) was found in BM21, it may contain an as-yet-undetermined NLS. The LRR motif has been found to be essential for nuclear localization of certain proteins. For example, the LRR motif in CIITA, the major histocompatibility complex class II transactivator, is found to be critical for the nuclear localization of CIITA (Hake *et al.*, 2000).
Alternatively, it has been suggested that phosphorylation in the vicinity of an NLS may play a role in the distribution of proteins (Jans & Hubner, 1996). BM21 is a leucine/isoleucine-rich protein (17.8 %) and 11 aa were predicted to be phosphorylated using NetPhos (data not shown). Therefore, the phosphorylation of BM21 may also have an effect on the nuclear transport.

Our results showed that a Bm21-null virus had growth curve dynamics similar to the parental virus (Fig. 5) and could cause mortality in infected larvae (Fig. 6 and Table 1). Therefore, Bm21 is not an essential gene for virus replication either in vitro or in vivo. A bioassay, however, showed that, although deletion of Bm21 did not reduce the LC50 of the virus, it did increase the ST50 of the infected larvae. Therefore, BM21 is directly or indirectly involved in the oral infectivity of the virus. So far, several baculovirus genes have been shown to be related to virus lethal time. For example, the baculovirus-encoded ecdysteroid UDP-glucosyltransferase (EGT) disrupts the hormone balance of the insect host by conjugation of ecdysteroids with galactose or glucose from UDP-galactose or UDP-glucose (O’Reilly & Miller, 1989). It has been demonstrated that deletion of the egt gene from certain baculovirus genomes increases the speed of killing of the baculoviruses (O’Reilly & Miller, 1991; Flipsen et al., 1995; Chen et al., 2000). Fibroblast growth factor gene (fgf) is conserved in baculoviruses (Katsuma et al., 2004) and is involved in efficient BV production and stimulation of migration of insect cells (Katsuma et al., 2006; Detvisitsakun et al., 2007). The deletion of fgf delayed the time of death of infected larvae (Katsuma et al., 2006; Detvisitsakun et al., 2007). Baculovirus chitinase is involved in the liquefaction of infected larvae. Deletion of the KDEL motif of the AcMNPV chitinase gene resulted in reduced LD50 and median lethal time (LT50) values of the recombinant virus in Trichoplusia ni larvae, and this was suggested to be associated with the redistribution of chitinase in infected cells (Saville et al., 2004). AC23 is a homologue of the envelope fusion protein in AcMNPV, and it has been shown that deletion of ac23 results in a significant delay in ST50 (Lung et al., 2003). Therefore, the ST50 of baculoviruses apparently can be influenced by multiple factors. As the in vitro infectivity of the Bm21-null virus was equivalent to the parental virus, Bm21 appears to influence a feature not seen in the environment of cell culture where a single-cell phenotype is present. Bm21 may influence intercellular spread, escape from the midgut or virus productivity in specific body cell types, for example, which affect the speed of virus infection. Exactly how Bm21 functions needs to be studied further. It should be noted that, as Bm21 is co-transcribed with Bm20, we cannot exclude subsequent changes in Bm20 that may influence the behaviour of the Bm21-null virus.

LRRs are generally 20–29 aa and contain a conserved 11 aa segment, LxxLxLxxN/CxxL (Kobe & Kajava, 2001). LRR proteins can be divided into seven subfamilies according to their structure (Kobe & Kajava, 2001). BM21 and its baculovirus homologues do not belong to any of the seven subfamilies as they have a different structure containing tryptophan repeats characterized as X-W-X2-L-X5-L-X4-L (Dall et al., 2001). As the LRRs in BM21 are not one of the seven LRR subfamilies (Fig. 1), it remains to be confirmed whether they are indeed LRR proteins. In addition to group I NPVs, Melanoplus sanguinipes entomopoxvirus and Amsacta moorei entomopoxvirus also contain LRR proteins with structures similar to BM21 (Afonso et al., 1999; Bawden et al., 2000; Dall et al., 2001). The existence of BM21 homologues in two different families of insect virus implies that the gene may play an important role in the infection cycles of certain insect viruses, and our bioassay result has shown that BM21 is somehow involved in infection of larvae.

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<th>Table 1. Bioassay results of vBmBacJS13-ph and vBmBacJS13A21-ph in third-instar B. mori larvae</th>
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Fig. 6. Mortality–time curves of vBmBacJS13-ph and vBmBac-JS13A21-ph in third-instar B. mori larvae.
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