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# Comparison of Promoter Activity of the p10 Gene between *Bombyx mori* Nucleopolyhedrovirus Variants

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**Abstract** To compare the p10 promoter activity of *Bombyx mori* nucleopolyhedrovirus (BmNPV) K1 and K4, recombinant viruses Bm101-LacZ and Bm104-LacZ with a *lac*Z gene under the control of each p10 promoter were constructed. The β-galactosidase activity due to Bm101-LacZ was about 5.5- and 1.1-fold higher than that due to Bm104-LacZ and BmK1-LacZ, respectively, expressing β-galactosidase under the control of a polyhedrin promoter. The recombinant virus BmK1-104LacZ with the same genome structure as Bm101-LacZ, except for a p10 promoter region, produced a similar β-galactosidase activity to that due to Bm104-LacZ and 5.5-fold lower than that due to Bm101-LacZ. The virus yield, expression level of polyhedrin, and polyhedra productivity for each recombinant virus was almost similar. These results suggested that the difference in the expression level of β-galactosidase resulted from a difference in the p10 promoter regions, and that an expression vector using the p10 promoter of BmNPV-K1 could be usefully exploited in the mass production of foreign proteins with silkworm larvae by means of oral ingestion.

**Key words:** *Bombyx mori* nucleopolyhedrovirus, p10 promoter, expression vector

Baculoviruses are a large group of insect DNA viruses with covalently closed dsDNA genomes. They are pathogenic for insects and used as biological insecticides [2]. Baculoviruses, notably *Autographa californica* nucleopolyhedrovirus (AcNPV) and *Bombyx mori* NPV (BmNPV), are also exploited as vectors for the high level expression of foreign genes [10, 11, 14].

During the very late phase of a baculovirus infection, two viral gene products, polyhedrin (the major constituent of polyhedra), and p10 are expressed in copious quantities. [20]. p10 is a major component of the so-called fibrillar structures in the infected cells [22] and is thought to play a role in disintegrating the nuclear matrix and supporting the release of polyhedra from cells and tissues [23]. Since the polyhedrin and p10 genes are under the control of very-late strong transcriptional promoters and are nonessential for virus replication, their promoters have been widely used for foreign gene expression [3, 6, 7, 9, 17, 30].

In both AcNPV and BmNPV expression vector systems, a polyhedrin promoter has been utilized for the expression of many foreign genes, whereas several foreign genes have recently been expressed using a p10 promoter [24, 25, 26]. Although the promoter activity of p10 is relatively lower than that of polyhedrin [15, 19, 21], utilization of a p10 promoter for foreign gene expression enables the recombinant virus to be occluded in polyhedral inclusion bodies and, therefore, readily infectious for insects when administered *per os* [25]. For the mass production of a foreign gene product, a BmNPV vector has the unique advantage of having an advanced system, i.e., the silkworm *B. mori*, which can be used for *in vivo* expression, and this system has several attractive features in comparison with an AcNPV vector system [11, 12, 18]. Therefore, the construction of occluded recombinant viruses using a p10 promoter is expected to increase the utility of the silkworm production system.

Recently, four BmNPV variants were isolated that have somewhat different characteristics, including different restriction enzyme patterns and different release rates of polyhedra from infected Bm5 cells [4]. In relation to the different release rates of polyhedra, the structures of the polyhedrin and p10 genes of these variants were compared. The results revealed that the p10 promoter structures of BmNPV-K1 and K4 were very different from each other. Accordingly, to study the difference in the p10 promoter activity of these two BmNPV variants, expression vectors were constructed using p10 promoters, then the expression efficiency of β-galactosidase under the control of these promoters was compared.

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## MATERIALS AND METHODS

### Cells and Viruses

The Bm5 cells were kindly provided by Dr. P. V. Choudary (Univ. of California at Riverside, U.S.A.) and maintained at 27°C in a TC-100 medium (Gibco-BRL, Rockville, MD, U.S.A.) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL, Rockville, MD, U.S.A.). The BmNPV-K1 and K4 were used as wild-type viruses [4]. The recombinant BmNPV, BmK1-LacZ containing the *E. coli lacZ* gene under the control of a polyhedrin promoter, was used in this study for comparison of the p10 and polyhedrin promoter activities [29]. The wild-type BmNPVs and recombinant viruses were propagated in Bm5 cells. Routine cell culture maintenance and virus production procedures were carried out according to previously published procedures [17].

### Polymerase Chain Reaction and Primers

A 20-mer oligonucleotide containing an *Eco*RV site at the 5' end (primer P1, 5'-GATATCATACA ATATGACTC-3') and 33-mer oligonucleotide containing a *Pst*I site at the 5' end (primer P2, 5'-CT GCAGTGATAGTAAATAAAATG-TAATTTACAG-3') were synthesized for the amplification of the p10 5'-flanking region including the p10 promoter of BmNPV-K4. Then, for the 3'-flanking region including the p10 gene termination region of BmNPV-K4, a 30-mer oligonucleotide containing a *Pac*I site at the 5' end (primer P3, 5'-TTAATTAATTAAAGCCTACGCTGAAAATCC-3') and 17-mer oligonucleotide containing a *Sac*II site at the 5' end (primer P4, 5'-CCGCGGTACCGAACGTG-3') were synthesized. In a polymerase chain reaction (PCR), the genomic DNA of the wild-type BmNPVs were subjected to 33 PCR cycles (1 min at 94°C, 1 min at 45°C, and 2 min at 72°C) followed by a 7 min final extension at 72°C using a Premix Top™ (Bioneer, Chungbuk, Korea) and DNA Thermal Cycler (Perkin Elmer, Norwalk, CT, U.S.A.).

### Construction of p10-Based Transfer Vectors from BmNPV-K1 and K4

To construct the p10-based transfer vectors, the p10 5'- and 3'-flanking regions were PCR amplified separately from BmNPV-K1 and K4 genomes. Two synthetic primers corresponding to −1,383~−1,364 and −26~+1 from the p10 translation start codon were used to amplify a 1.4 kb 5'-flanking fragment of BmNPV-K1. In the case of BmNPV-K4, two synthetic primers corresponding to −1,383~−1,364 and 26~+1 were used for amplification of the 1.4 kb 5'-flanking fragment. These amplified fragments were cloned into a pGemT-easy vector (Promega, Madison, WI, U.S.A.), cleaved with *Eco*RV and *Pst*I, and then cloned again into a pBluescriptIIKS(+) vector (Stratagene, La Jolla, CA, U.S.A.) digested with the same restriction endonucleases (Fig. 2). For the 3'-flanking region, two synthetic primers corresponding to +212~+236 and +1,317~+1,333 were used for amplification of a 1.1-kb 3'-flanking fragment of BmNPV-K1, and two synthetic primers corresponding to +211~+231 and +1,317~+1,333 were exploited to amplify the 1.1-kb 3'-flanking fragment of BmNPV-K4. The amplified 3'-flanking fragments were cloned into pGemT-easy and then cleaved with *Spe*I and *Sac*II (Fig. 2). These 1.1-kb 3'-flanking fragments were ligated just downstream of the 1.4-kb 5'-flanking fragments of each virus to create the transfer vectors pBm101 and pBm104 (Fig. 2).

### Construction of Recombinant BmNPVs Containing the *lacZ* Gene

A 3.7-kb fragment containing the *lac*Z gene was isolated from the plasmid pCH110 (Amersham Pharmacia Biotech. Uppsala, Sweden), which was digested with *Hin*dIII, blunted with the Klenow fragment, and then cleaved with *Bam*HI. This 3.7-kb fragment was cloned into pBm101 and pBm104, which were then digested with *Sma*I and *Bam*HI (Fig. 2). The resulting plasmids, pBm101-LacZ and pBm104-LacZ, were independently transfected with the corresponding wild-type viral genomic DNA into Bm5 cells to construct the recombinant viruses, Bm101-LacZ and Bm104-LacZ, where the p10 gene was replaced with the *lac*Z gene. The recombinant virus, BmK1-104LacZ, was constructed by cotransfection of pBm104-LacZ with the genomic DNA of wild-type BmNPV-K1.

### Transfection and Selection

Bm5 cells in a 60-mm tissue culture dish were cotransfected with wild-type BmNPV DNAs and transfer vectors by the liposome-mediated method using the transfection reagent Lipofectin™ (Gibco-BRL, Rockville, MD, U.S.A.). The transfection supernatants were subjected to an end-point dilution [17] in order to obtain the recombinant viruses. The end-point dilution was performed three times to clone the viruses genetically. The recombinant viruses were propagated and stored at −20°C.

### B-Galactosidase Assay and SDS-PAGE

The Bm5 cells were seeded into 6-well tissue culture plates at a density of $1\times10^6$ cells/well and allowed to attach for 1 h. The cells were inoculated with the recombinant viruses at a multiplicity of infection (MOI) of 10 plaque forming units (PFU)/cell in 1 ml of a serum-free medium. After 2 h, the medium was replaced with 3 ml of a fresh medium. The total protein contents of virus-infected cells at 3 days p.i. were analyzed by SDS-PAGE. The total β-galactosidase activities including the extracellular and intracellular activities were determined at various time points (0–6 days) after infection by the recombinant viruses. The β-galactosidase activity was measured by an ONPG assay using a β-galactosidase enzyme assay system (Promega, Madison, WI, U.S.A.) and microplate reader model 550 (BIO-RAD, Hercules, CA, U.S.A.).

### Polyhedra Production and Virus Titration

The Bm5 cells ($1\times10^6$ cells) were infected with each recombinant virus at a MOI of 10 PFU/cell. For quantification of the polyhedral inclusion bodies (PIBs), 1% sodium dodecyl sulfate was added to the infected cultures 6 days post infection (p.i.) to release the PIBs, which were then incubated at 37°C for 30 min. The released PIBs were quantified using a hemocytometer. The end-point dilution for the titration of the virus stocks was carried out according to a previously published procedure [17].

## RESULTS AND DISCUSSION

### P10 Promoter Structure of BmNPV Variants

The p10 promoter sequence of BmNPV-K1 (GenBank accession number AF247681) was different from that of BmNPV-K4 (GenBank accession number AF247684) at seven base sequences (Fig. 1). The p10 promoter sequence of BmNPV-K1 was also different from those of BmNPV, as reported previously by Hu *et al.* [5], Tomita *et al.* [21], and Zhang *et al.* [31], at seven or eight base sequences. In contrast, the p10 promoter sequence of BmNPV-K4 was identical with that reported by Tomita *et al.* [21], and only different from those reported by Hu *et al.* [5] and Zhang *et al.* [31] at one and two base sequences, respectively. Therefore, the activities of the p10 promoters were compared based on their different structures.

### Construction of Transfer Vectors and Recombinant Viruses

To compare the p10 promoter activity of BmNPV-K1 with that of BmNPV-K4, a transfer vector was constructed to permit the insertion of foreign genes into the virus genome

```
BmNPV-K1   -101   TTCAACCCAACACAATATATTATAGTTAAATAAGA   -67
BmNPV-K4   -101   ····················C··C·········   -67
BmNPV-H    -101   ····G···············C··C·········   -67
BmNPV-Z    -101   ····G···············C··C·········   -67
BmNPV-T    -101   ····················C··C·········   -67

BmNPV-K1    -66   ATTATTATCAAATCATTTGTATATTAATTAAAATA   -32
BmNPV-K4    -66   ·····T····T·····················-   -33
BmNPV-H     -66   ·····T····T·····················-   -33
BmNPV-Z     -66   ·····T····T·····················-   -33
BmNPV-T     -66   ·····T····T·····················-   -33

BmNPV-K1    -31   C-TATACTGTAAATTACATTTTATTTACAATCATG   +3
BmNPV-K4    -32   ·T·····························T···ATG   +3
BmNPV-H     -32   ·T·····························T···ATG   +3
BmNPV-Z     -32   ·T·····························ATG   +3
BmNPV-T     -32   ·T·····························T···ATG   +3
```

**Fig. 1.** Nucleotide sequences of p10 promoters of BmNPV-K1 and K4.

The nucleotide sequences of the p10 promoter of the BmNPV variants were compared with that of BmNPV as reported by Hu *et al.* (1994, BmNPV-H), Zhang *et al.* (1995, BmNPV-Z), and Tomita *et al.* (1995, BmNPV-T).

under the control of a p10 promoter, in lieu of a p10 coding sequence (Fig. 2). The pBm101 and pBm104 originated from the p10 genes of BmNPV-K1 and K4, respectively. The p10 promoter sequences in pBm101 and pBm104 were confirmed by nucleotide sequence determination. As a result, these new transfer vectors were found to have unique *Pst*I, *Sma*I, *Bam*HI, *Spe*I, and *Pac*I sites for the insertion of foreign genes immediately downstream of the p10 promoter, plus the p10 coding genes were deleted from +2 to +211/+210.

For a comparative study of the p10 promoter activity of BmNPV-K1 and K4, the recombinant viruses, Bm101-LacZ and Bm104-LacZ, harboring the *lac*Z gene under the control of a p10 promoter were constructed (Fig. 2). The recombinant viruses producing β-galactosidase were identified as blue plaques when incubated with X-gal. Following purification, the purity of the recombinant viruses was confirmed by an analysis of the viral genomic DNAs using a PCR with primers specific to *lac*Z and p10 genes, respectively (data not shown).

### Expression of β-Galactosidase in Bm5 Cells Infected with Recombinant BmNPVs

The protein accumulation in the Bm5 cells infected with Bm101-LacZ and Bm104-LacZ 3 days p.i. was analyzed by SDS-PAGE. When the cells were infected with the recombinant viruses, a new protein band of approximately 116 kDa for β-galactosidase was observed (Fig. 3). This new protein band was absent in both the mock-infected and wild-type BmNPV-K1- or BmNPV-K4-infected cells. The β-galactosidase activities in the Bm5 cells infected with the recombinant viruses were compared with each other (Fig. 4). When assayed at 1-day intervals over 6 days, the β-galactosidase expression peaked 5 days p.i. with all viruses. The β-galactosidase activity with infection by Bm101-LacZ was 5.5 times higher than that by Bm104-LacZ. In particular, the β-galactosidase activity with cells infected by Bm101-LacZ was about 10% higher than that by BmK1-LacZ, which was the recombinant BmNPV expressing the *lac*Z gene under the control of a polyhedrin promoter.

BmNPV-K1 and K4 showed differences not only with a p10 promoter, but also in their other genomic structures, as revealed by a restriction endonuclease analysis [4]. To address the effects of other genomic factors on the expression of the *lac*Z gene, the recombinant virus BmK1-104LacZ was constructed by the cotransfection of pBm104-LacZ with the genomic DNA of BmNPV-K1. When the β-galactosidase expression efficiency was compared, the β-galactosidase activity due to BmK1-104LacZ was basically similar to that due to Bm104-LacZ and about 5.5 times lower than that due to Bm101-LacZ (Fig. 4).

This strongly suggests that the difference in the expression level of β-galactosidase resulted from the p10 promoter

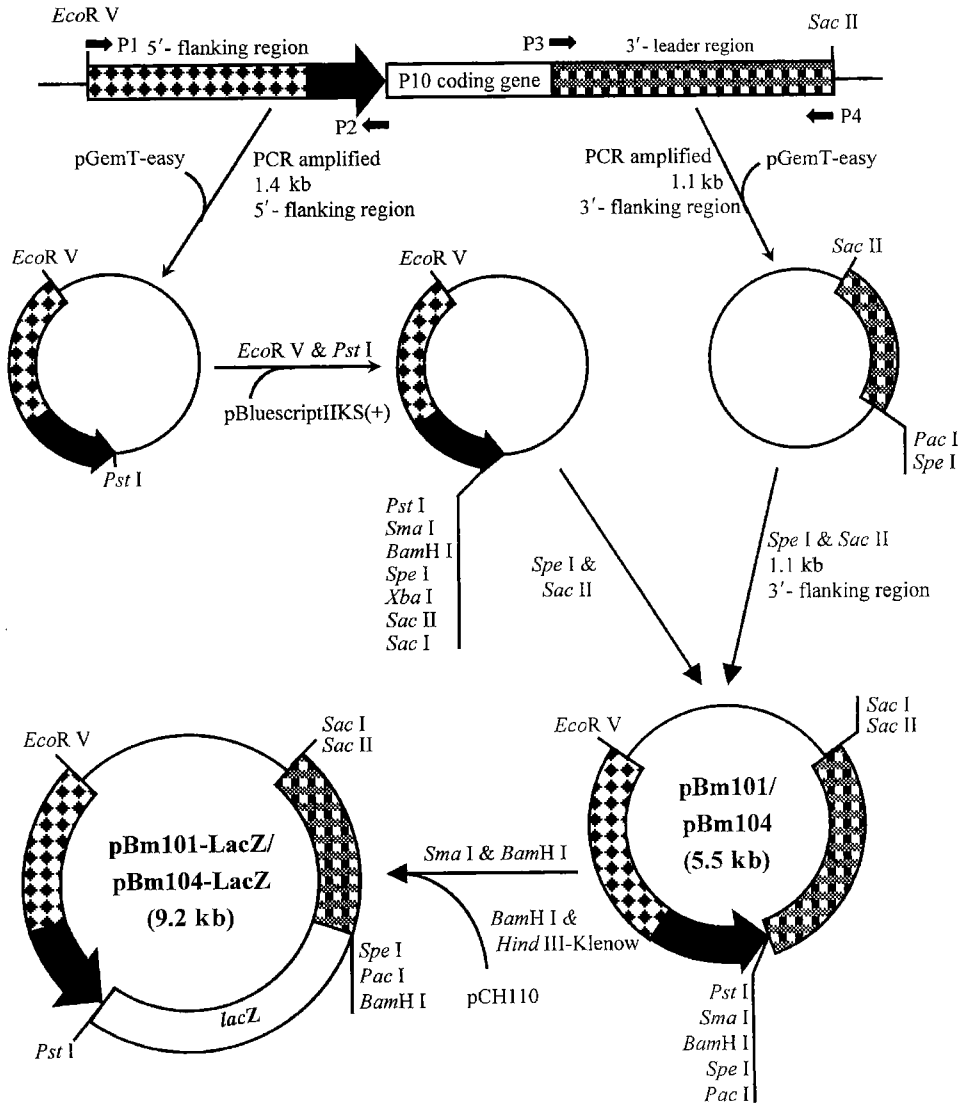


**Fig. 2.** Schematic diagram of transfer vector construction using the p10 promoter.
The 5'- and 3'- flanking regions were cloned using the PCR technique. The solid arrows show the direction of transcription.

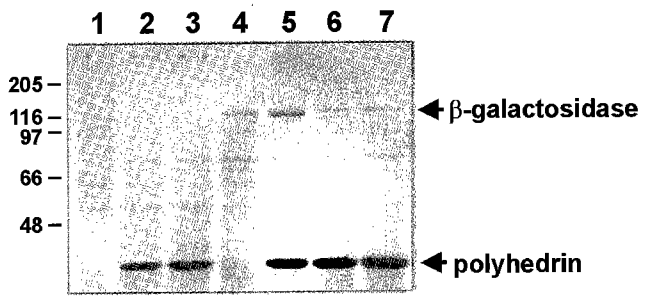


**Fig. 3.** SDS-PAGE of β-galactosidase protein from Bm5 cells after mock-infection or virus infection.
Proteins were isolated 3 days p.i., electrophoresed on 10% SDS-polyacrylamide gel. Positions of the protein molecular mass standards are indicated on the left in kilodaltons. Lanes: 1, mock-infected cells; 2, BmNPV-K1; 3, BmNPV-K4; 4, BmK1-LacZ; 5, Bm101-LacZ; 6, Bm104-LacZ; 7, BmK1-104LacZ.

structure. Of course, maximum p10 expression requires the integrity of the complete 5' untranslated region (UTR) plus several upstream nucleotides [27, 28]. The 3' UTR and polyadenylation signals may also effect the efficiency of p10 mRNA generation and its stability [23]. Previously, it was reported that the promoter activity of p10 appears to be relatively lower than that of polyhedrin. These results were obtained in comparative studies on the promoter functions between the p10 and polyhedrin of AcNPV and BmNPV [15, 19, 21, 26]. However, in this study, the β-galactosidase activity due to Bm101-LacZ was about 10% higher than that due to BmK1-LacZ, thereby indicating that the activity of the p10 promoter of BmNPV-K1 was higher than that of the polyhedrin promoter of the same virus (Fig. 2). To address the reason for this result, further

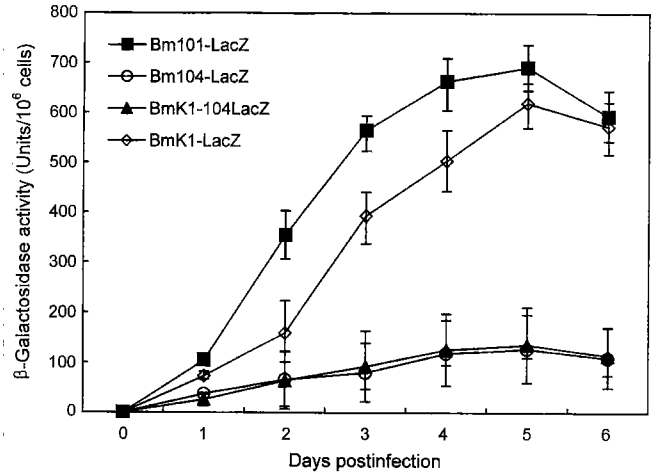


**Fig. 4.** β-Galactosidase activity in Bm5 cells infected with recombinant BmNPVs.
The cells were seeded at a density of $1\times10^6$ cells/well and inoculated with viruses at an MOI of 10 PFU/cell. The culture media and cells were harvested every day for 6 days and the total β-galactosidase activities were determined. Data for each time point were derived from three independent sets of infected cells.

investigation concerning the regulation and transcription from these promoters is needed.

## Replication and Polyhedrin Expression of Recombinant BmNPVs

In order to address the relation of the expression efficiency and the viral replication of the recombinant viruses, the titers of the recombinant viruses were determined 5 days p.i. (Fig. 5A). The viral titers of Bm101-LacZ, Bm104-LacZ, and BmK1-104LacZ were almost the same, and these titers were lower than that of BmK1-LacZ, which did not produce polyhedra. This result also supports the difference in the expression level of β-galactosidase resulted from the p10 promoter structure. Since BmK1-LacZ is a polyhedrin-negative virus, it is believed that the higher replication of BmK1-LacZ resulted from a lack of occluded virus formation.

The PIB levels of Bm101-LacZ, Bm104-LacZ, and BmK1-104LacZ formed in the Bm5 cells were also determined 5 days p.i. (Fig. 5B). In contrast to the expression efficiency of β-galactosidase, the PIB levels of each recombinant viruses were very similar with each other, and the polyhedrin expression levels of all the recombinant viruses were not only similar with each other, (Fig. 3) but also to those for the wild-type viruses BmNPV-K1 and K4 (data not shown). These results have been described in a previous report [4], in that the polyhedrin gene region of these two isolates was the same. This suggested that an expression vector system using the p10 promoter of BmNPV-K1 could be usefully exploited for the mass production of foreign proteins with silkworm larvae by means of oral infection.

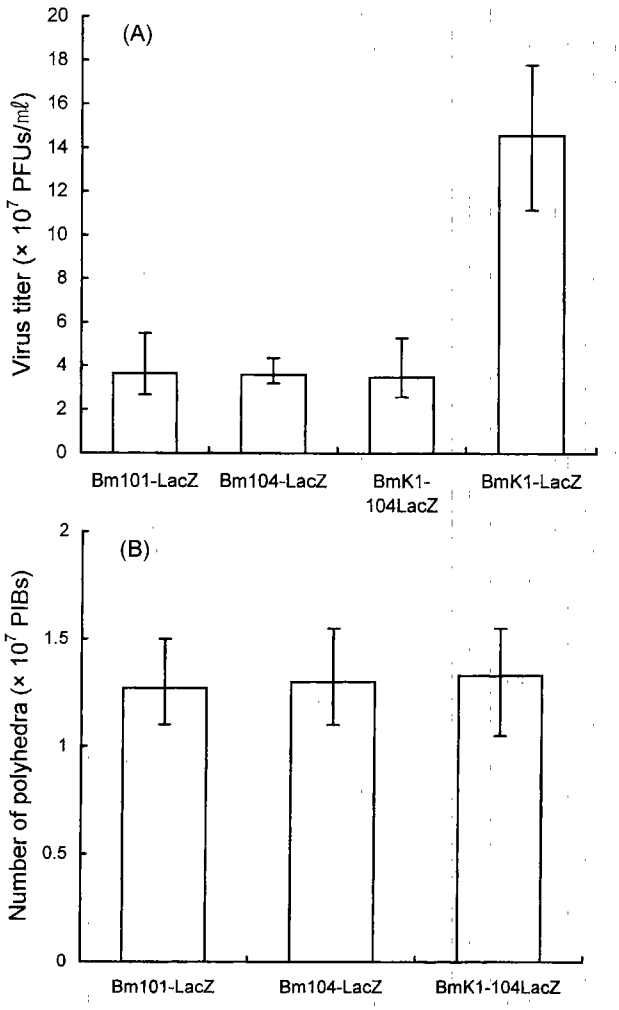


**Fig. 5.** Titration and PIB production of recombinant BmNPVs in Bm5 cells.
Cells ($1\times10^6$) were infected with each virus at an MOI of 10 PFU/cell. (A) The extracellular virus production was determined by an end-point dilution assay 5 days p.i. (B) The cells were harvested 5 days p.i., lyzed, and three independent polyhedra counts made using a hemocytometer.

In a baculovirus expression vector system, several foreign genes have been expressed using a p10 promoter [24, 25, 26]. The utilization of a p10 promoter for foreign gene expression enables recombinant viruses to be occluded in polyhedral inclusion bodies. Also, a BmNPV vector system has the unique advantage of expressing foreign genes in silkworm larvae on a large scale. Previous studies have demonstrated that the expressions of some proteins, such as human α-interferon [13], interleukin-3 [16], α-amidating enzyme [8], and canine parvovirus VP2 coat protein [1] are significantly higher in concentration in the hemolymph of silkworm larvae than in the culture supernatant or lysate of insect cells.

In this study, the p10 promoter of BmNPV-K1 was found to be a very effective promoter for the high level expression of foreign genes. In addition, recombinant

viruses constructed with p10-based transfer vectors formed occluded viruses, and thus could be used easily through oral ingestion for infecting a large number of silkworm larvae.

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## REFERENCES

1. Choi, J. Y., S. D. Woo, H. K. Lee, H. K. Hong, Y. H. Je, J. H. Park, J. Y. Song, S. H. An, and S. K. Kang. 2000. High-level expression of canine parvovirus VP2 using *Bombyx mori* nucleopolyhedrovirus vector. *Arch. Virol.* **145:** 171–177.
2. Granados, R. R. and B. A. Federici. 1986. *The Biology of Baculoviruses.* CRC Press, Boca Raton, U.S.A.
3. Han, B. K., B. Y. Lee, M. K. Min, and K. H. Jung. 1998. Expression and characterization of recombinant E2 protein of Hepatitis C virus by insect cell/baculovirus expression system. *J. Microbiol. Biotechnol.* **8:** 361–368.
4. Hong, H. K., S. D. Woo, J. Y. Choi, H. K. Lee, M. H. Kim, Y. H. Je, and S. K. Kang. 2000. Characterization of four isolates of *Bombyx mori* nucleopolyhedrovirus. *Arch. Virol.* **145:** 2351–2361.
5. Hu, N. T., Y. F. Lu, Y. Hashimoto, S. Maeda, and R. F. Hou. 1994. The p10 gene of natural isolates of *Bombyx mori* nuclear polyhedrosis virus encodes a truncated protein with an Mr of 7700. *J. Gen. Virol.* **75:** 2085–2088.
6. Kang, D. K., K. W. Kim, P. H. Kim, S. Y. Seoung, Y. H. Kim, I. C. Kwon, S. Y. Jeong, E. Y. Choi, K. M. Lee, H. S. Kim, E. C. Kim, S. I. Joo, and J. M. Yang. 1998. cDNA cloning and expression of human rotavirus outer capsid protein VP7 in insect cells. *J. Microbiol. Biotechnol.* **8:** 369–377.
7. King, L. A. and R. D. Possee. 1992. *The Baculovirus Expression System: A Laboratory Guide.* Chapman and Hall, London, U.K.
8. Kobayashi, J., S. Imanishi, H. Inoue, K. Oshue, K. Yamaichi, N. Tsuruoka, and S. Tanaka. 1992. High level expression of a frog α-amidating enzyme, AE-II, in cultured cells and silkworm larvae using a *Bombyx mori* nuclear polyhedrosis virus expression vector. *Cytotechnology* **8:** 103–108.
9. Koh, Y. W., S. K. Park, K. S. Kim, S. R. Kim, J. K. Lee, and J. M. Yang. 1999. Expression of human thrombopoietin in insect cells by polyhedrin-gp64 dual promoter-based baculovirus vector system. *J. Microbiol. Biotechnol.* **9:** 318–322.
10. Luckow, V. L. and M. D. Summers. 1988. Trends in the development of baculovirus expression vectors. *Bio/Technology* **6:** 47–55.
11. Maeda, S. 1989. Expression of foreign genes in insects using baculovirus vectors. *Annu. Rev. Entomol.* **34:** 351–372.
12. Maeda, S. 1994. Expression of foreign gene in insect cells using baculovirus vectors, pp. 1–31. *In* K. Maramorosch and A. McIntosh (eds.), *Insect Cell Biotechnology.* CRC Press, Boca Raton, U.S.A.
13. Maeda, S., T. Kawai, M. Obinata, H. Fujiwara, T. Horiuchi, Y. Saeki, Y. Soto, and M. Furusawa. 1985. Production of human α-interferon in silkworm using a baculovirus vector. *Nature* **315:** 592–594.
14. Miller, L. K. 1988. Baculoviruses as gene expression vectors. *Annu. Rev. Microbiol.* **42:** 177–199.
15. Min, M. K. and D. H. L. Bishop. 1991. Transcriptional analyses of baculovirus polyhedrin and foreign gene expression relative to baculovirus p10 mRNA levels. *J. Gen. Virol.* **72:** 2551–2556.
16. Miyajima, A., J. Scheurs, K. Otsu, A. Kondo, K. Arai, and S. Maeda. 1987. Use of silkworm *Bombyx mori,* and insect baculovirus vector for high-level expression and secretion of biologically active mouse interleukin-3. *Gene* **58:** 273–281.
17. O'Reilly, D. R., L. K. Miller, and V. A. Luckow. 1992. *Baculovirus Expression Vectors: A Laboratory Manual.* W. H. Freeman and Company, New York, U.S.A.
18. Reis, U., B. Blum, B. R. von Specht, H. Domdey, and J. Collins. 1992. Antibody production in silkworm cells and silkworm larvae infected with a dual recombinant *Bombyx mori* nuclear polyhedrosis virus. *Bio/Technology* **10:** 910–912.
19. Roelvink, P. W., M. M. M. van Meer, C. A. D. de Kort, R. D. Possee, B. D. Hammock, and J. M. Vlak. 1992. Dissimilar expression of *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus polyhedrin and p10 genes. *J. Gen. Virol.* **73:** 1481–1489.
20. Smith, G. E., J. M. Vlak, and M. D. Summers. 1982. *In vitro* translation of *Autographa californica* nuclear polyhedrosis virus early and late mRNAs. *J. Virol.* **44:** 199–208.
21. Tomita, S., T. Kanaya, J. Kobayashi, and S. Imanishi. 1995. Isolation of p10 gene from *Bombyx mori* nuclear polyhedrosis virus and study of its promoter activity in recombinant baculovirus vector system. *Cytotechnology* **17:** 65–70.
22. van Oers, M. M., J. T. M. Flipsen, C. B. E. M. Reusken, E. L. Sliwinsky, R. W. Goldbach, and J. M. Vlak. 1993. Functional domains of the p10 protein of *Autographa californica* nuclear polyhedrosis virus. *J. Gen. Virol.* **74:** 563–574.
23. van Oers, M. M. and J. M. Vlak. 1997. The baculovirus 10-kDa protein. *J. Invertebr. Pathol.* **70:** 1–17.
24. Vialard, J., M. Lalumiere, T. Vernet, D. Briedis, G. Alkhatib, D. Henning, D. Levin, and C. Richardson. 1990. Synthesis of the membrane fusion and hemagglutinin proteins of measles virus, using a novel baculovirus vector containing the β-galactosidase gene. *J. Virol.* **64:** 37–50.
25. Vlak, J. M., A. Schouten, M. Usmany, G. J. Belsham, E. C. Klinge-Roode, A. J. Maule, J. W. M. van Lent, and D. Zuidema. 1990. Expression of cauliflower mosaic virus gene using a baculovirus vector based upon the p10 gene and a novel selection method. *Virology* **179:** 312–320.

26. Weyer, U., S. Knight, and R. D. Possee. 1990. Analysis of very late gene expression by *Autographa californica* nuclear polyhedrosis virus and the further development of multiple expression vectors. *J. Gen. Virol.* **71:** 1525–1534.
27. Weyer, U. and R. D. Possee. 1988. Functional analysis of the p10 gene 5' leader sequence of the *Autographa californica* nuclear polyhedrosis virus. *Nucleic Acids Res.* **16:** 3635–3653.
28. Weyer, U. and R. D. Possee. 1989. Analysis of the promoter of the *Autographa californica* nuclear polyhedrosis virus p10 gene. *J. Virol.* **70:** 203–208.
29. Woo, S. D., W. J. Kim, H. S. Kim, B. R. Jin, and S. K. Kang. 1996. Expression of *Escherichia coli* β-galactosidase gene by new transfer vector of baculovirus. *Kor. J. Appl. Microbiol. Biotechnol.* **24:** 72–76.
30. Yoo, D. W., J. C. Graham, and I. D. Yoo. 1992. Phosphorylation of nucleocapsid protein of bovine coronavirus expressed with a recombinant baculovirus vector. *J. Microbiol. Biotechnol.* **2:** 122–128.
31. Zhang, Y., X. Wu, and Z. Li. 1995. P10 genes of *Bombyx mori* nuclear polyhedrosis virus and *Autographa californica* multiple nuclear polyhedrosis virus. *Sci. China B, Chem. Life Sci. Earth Sci.* **38:** 50–59.