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Isolation and Characterization of *Plutella xylostella* Granulovirus Isolated in Korea

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We have first isolated and characterized a *Plutella xylostella* granulovirus (PxGV) from dead larvae of *P. xylostella* in Korea. The granule of PxGV was ovoidal shape with an approximate measure of 350 – 400 nm × 150 – 200 nm, and each granule contained one single rod-shape virion with a mean size of 150 – 180 nm × 20 – 30 nm. The major granule protein, granulin, had a molecular weight of approximately 29 kDa. Whereas the nucleotide sequence of the granulin gene was identical to that of previously reported PxGV, nucleotide sequences of two of three putative p10 genes were slightly different from those of reported PxGV. These results suggested that the PxGV isolated in this study was a novel isolate containing different genomic information.

Key words: Bculovirus, *Plutella xylostella* granulovirus, Granulin gene, p10 gene

Introduction

The diamondback moth, *Plutella xylostella* is a widely-distributed and serious pest of crucifers in subtropical areas including South Asia, South-East Asia and South America (Abdul Kadir *et al.*, 1999a). In most regions of the world where *P. xylostella* is a pest, control has been heavily dependent on chemical pesticides although some progresses have been made with biological and integrated pest management approaches. The heavy dependence on chemical pesticides has created severe pesticide resistance problems (Abdul Kadir *et al.*, 1999a). These severe pesticide resistance problems have prompted considerable interest in biological methods for *P. xylostella* control, including the introduction and manipulation of parasitoids and pathogens, such as *Bacillus thuringiensis* (Zhao *et al.*, 2000). In recent years, *B. thuringiensis* products have been widely used for *P. xylostella* control but genetic resistance in populations to some *B. thuringiensis* strains, compounded by cross-resistance to several different *B. thuringiensis* toxins, has also been identified (Tabashnik *et al.*, 1997). Such recent resistance problems serve to emphasize the urgent need for alternative control agents and their use within an integrated pest management approach.

Baculoviruses have been used as agents for the biological control of certain insect pest species (Zhang *et al.*, 2005). Two different members have been accepted within the baculoviruses, nucleopolyhedroviruses (NPVs) and the granuloviruses (GVs), based on the structure of the occluded virus and the occlusion body (OB). Most of our knowledge on the baculoviruses relates to the NPVs. The GVs are less studied because of the limited availability of permissive insect cell lines. Several baculoviruses including *Autographa californica* NPV (AcNPV) and *Galleria mellonella* NPV (GmNPV) have been reported to be infective to *P. xylostella* (Vail *et al.*, 1972; Abdul Kadir *et al.*, 1999a, 1999b). Since GV infecting *P. xylostella* (PxGV) was first reported by Asayama and Osaki (1970), several reports have showed PxGV as a promise control agent for *P. xylostella* (Kao and Rose, 1976; Wang and Rose, 1978).

Although the complete nucleotide sequence of PxGV

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genome has been reported (Yoshifumi *et al.*, 2000), no research has been performed on characteristics of this virus. In this study, a PxGV isolated from Korea was identified and characterized.

Materials and Methods

Virus isolate

Homogenate of PxGV-infected *P. xylostella* larvae was filtered through sterile cheesecloth. The mixture was centrifuged at 5,000 × g for 5 min, and then, the pellets were washed three times with distilled water. The viral occlusion bodies were layered onto 40% – 65% discontinuous sucrose gradient and were centrifuged at 80,000 × g for 60 min. The OBs were collected by centrifugation at 10,000 × g for 10 min. Then, they were re-suspended with 0.5 M NaCl (Shim *et al.*, 2003).

Electron microscopy

For scanning electron microscopy, the purified OBs were dried at the critical point in CO_2. The samples were sputtered with gold in a sputter coater SC502 (Polaron, UK) and observed using field emission scanning electron microscope JSM-6700F (JEOL, Japan).

For transmission electron microscopy, the purified OBs were fixed for 2 hrs by 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2). After post-fixation by 1% OsO_4 in the same buffer, the samples were dehydrated in ethanol/propylene oxide series and embedded in Epon-Araldite mixture. Ultra-thin sections were performed with a RMC MT-X ultramicrotome and photographed under the transmission electron microscope JEM-1010 (JEOL, Japan).

SDS-PAGE

The OBs were mixed with the 5 × sample buffer (60 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 14.4 mM β-mercaptoethanol, 0.1% bromophenol blue) and were boiled for 5 min and were clarified by centrifugation (15,000 × g for 5 min). The total cellular lysates were separated on a 10% polyacrylamide gel containing SDS as described by Laemmli (1970).

PCR and oligonucleotide primers

The degenerate primers, Gr-F (5’-ATGGGATAYAACARAKCWYTRMGKTAYAGYMRHCAC-3’) and Gr-R (5’-TTARTAVGCBGGDCCDGTRWAYARWGGYACRTC-3’), specific to granulin gene were designed based on the nucleotide sequences of previously reported granulin genes. Three pairs of oligonucleotide primers, Pxorf2-F (5’-CAAACGTCACATAATGC-3’) and Pxorf2-R(5’-TGTTGCTGAAACTGTTC-3’), Pxorf21-F (5’-TCGATAACATGTCCAGA-3’) and Pxorf21-R (5’-TCTAGAATCTGCGCATA-3’), and Pxorf50-F (5’-CACAACGTGTATCTGGA-3’) and Pxorf50-R (5’-GGGTCGATTTACGATTT-3’), specific to three putative p10 genes of PxGV were also designed based on the nucleotide sequences of these genes reported by Yoshifumi *et al.* (2000). The PCR reaction was carried out with 250 ng of plasmid DNA, 100 pM of each primer and PCR PreMix (Bioneer Co., Korea) in a 20 µl PCR mixture for 33 thermal cycles (94°C for 1 min, 45°C for 1 min and 72°C for 1 min). Amplification was accomplished with the DNA Thermal Cycler (Perkin Elmer Cetus, USA).

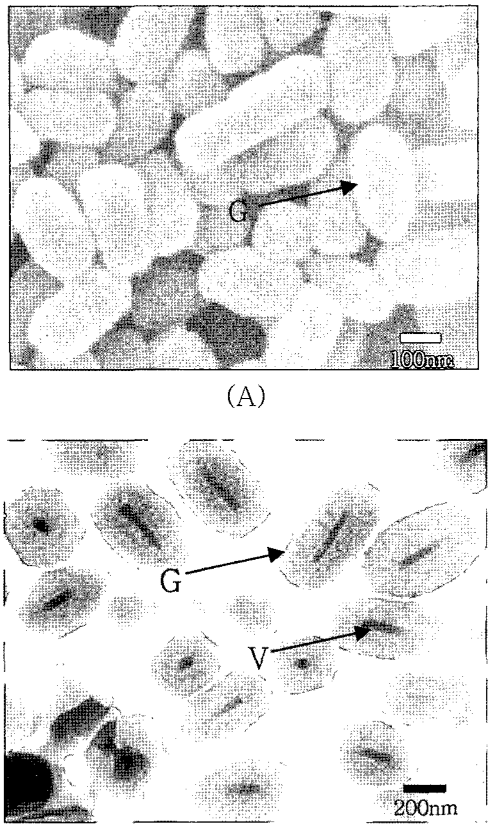
Cloning and sequence analysis

PCR amplified granulin and three p10 genes were cloned into pGEM-T Easy vector (Promega, USA), and their nucleotide sequences were determined on an ABI sequence Model 377 (ABI system, USA). The obtained sequences were compared with those of previously reported PxGV granulin gene and p10 genes using the BLAST.

Results and Discussion

In this study, we isolated PxGV from dead larvae of *P. xylostella*, and named PxGV-K1. *P. xylostella* larvae infected with PxGV showed very distinct symptoms, exhibiting puffy, elongated integument and a change of color from dull green to pale yellow as previously reported (Asayama and Osaki, 1970). The gross pathologies of granuloviruses are similar to those of nuclear polyhedroses, but differences occur depending on the types of tissues infected. The first indication of infection in the larva is a loss of appetite and a progressive in the ventral side (Huger, 1963). The whiteness is due to the abundance of capsules in the hypertrophied fat bodies. When the infection is limited mainly to the fat body, the larva often increases in size, becomes white, opaque, and mottled at an advanced stage of infection, and later has a brownish discoloration (Hamm and Paschke, 1963). Infected larvae may live longer and become larger than an uninfected one. With the change in color, infected larvae usually become progressively weaker, sluggish, and flaccid.

The granule of the PxGV was observed as elliptical shape, but the shapes varied greatly (Fig. 1A), which are general features of GV (Tanada and Kaya, 1993). The approximate measure of the granule was 350 – 400 nm × 150 – 200 nm. Transmission electron microscopy revealed that each granule contained single rod-shaped virion with



(A)

(B)

Fig. 1. Scanning (A) and transmission (B) electron micrographs of the granules of PxGV-K1. The granules were purified by ultracentrifugation in linear 40% to 65% sucrose density gradients. Granule and virion were indicated as G and V, respectively.

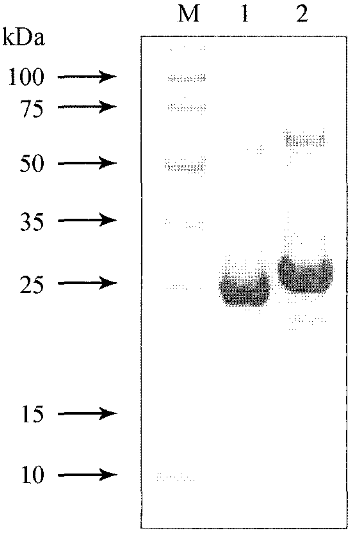


Fig. 2. SDS-polyacrylamide gel electrophoresis of the granulin protein of the PxGV-K1. Lane M: protein molecular weight marker; 1: granulin of PxGV-K1; 2: polyhedrin of AcNPV.

approximate size of 150 – 180 nm × 20 – 30 nm (Fig. 1B).

Granule of GV consisted of a major occlusion body protein, granulin, with a size of 27 – 31 kD (Tweeten *et al.*, 1981; Rohrmann, 1992). Granulin of PxGV-K1 was about 29 kDa, which was estimated by the SDS-PAGE electrophoresis and smaller than polyhedrin of AcNPV (31 kDa) (Fig. 2). This result was consistent with previous reports that molecular weight of granulins were about 27 – 31 kDa (Tweenten *et al.*, 1981; Rohman, 1992).

The nucleotide sequence of PxGV-K1 granulin gene was completely same with that of previously reported PxGV ganulin (Yoshifumi *et al.*, 2000). Whereas, when

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                   M   S  R  L   I  F  S  T   R  V  D   G  T  D   V  P  V  F
PxGV-K1    TCGATAACAT GTCCAGACTT ATATTTTCGA CGCGTGTGGA CGGCACCGAT GTCCCGGTTT    60
AF270937   .......... .......... .......... .......... .......... ..........
             F  G  G   A  P  T   D  K  P  Y   V  G  V   S  E  L   L  N  I  L
PxGV-K1    TTTTTGGTGG TGCACCCACA GATAAGCCCT ATGTGGGAGT CAGTGAGCTT TTGAACATTT   120
AF270937   .......... .......... .......... .......... .......... ..........
             G  H  S   K  S  H   A  E  E  F   P  R  S   E  T  K   L  W  S  E
PxGV-K1    TGGGACACAG CAAGTCTCAC GCCGAGGAGT TCCCCAGGAG CGAAACTAAA CTATGGTCCG   180
AF270937   .......... .......... .......... .......... .......... ..........
             L  A  P   G  D  T   T  F  P  A   N  K  L   F  T  T   E  V  G  F
PxGV-K1    AGTTGGCCCC CGGAGACACC ACGTTCCCCG CCAACAAGCT CTTTACCACC GAGGTCGGGT   240
AF270937   .A........ .......... .......... .......... .......... ..........
             A  V  Y   F  G  K   T  K  L  N   N  W  A   C  F  K   R  M  F  D
PxGV-K1    TCGCCGTGTA CTTTGGGAAA ACAAAGCTCA ATAATTGGGC GTGCTTTAAG CGCATGTTTG   300
AF270937   .......... .......... .......... .......... .......... ..........
             T  I  E   S  Y  I   L  N  P  A   G  C  T   A  T  N   P  L  C  M
PxGV-K1    ACACTATTGA ATCCTACATT TTAAACCCCG CAGGGTGCAC GGCCACAAAC CCCCTGTGTA   360
AF270937   .......... .......... .......... .......... .......... ..........
             I  P  P   G  F  N   T  G  C  G   S  N  P   C  N  P   N  N  G  D
PxGV-K1    TGATTCCCCC GGGGTTCAAC ACCGGCTGCG GATCCAACCC TTGCAACCCC AACAACGGGG   420
AF270937   .......... .......... .......... .......... .......... ..........
             L  G  Q   V  L  A   L  L  Q  Q   I  L  A   I  I  S   N  P  N  P
PxGV-K1    ACCTCGGACA GGTTTTGGCT CTGTTGCAGC AAATTTTAGC AATAATATCT AACCCCAACC   480
AF270937   .......... .......... .......... .......... ...C...... ..........
             T  N  P   V  D  L   T  P  V  L   D  A  I   A  A  L   A  A  Q  V
PxGV-K1    CCACCAACCC CGTGGATCTG ACTCCGGTTC TCGACGCCAT AGCCGCTCTC GCCGCTCAGG   540
AF270937   .......... .......... .......... .T........ .......... ..........
             T  A  L   T  T  T   V  N  T  I   N  D  N   V  T  A   L  D  G  R
PxGV-K1    TCACCGCGCT CACCACCACC GTCAACACGA TAAACGATAA CGTCACCGCT CTCGACGGCA   600
AF270937   .......... .......... .......... .......... .......... ..........
             I  T  N   L  E  T   T  L  N  N   F  V  T   T  T  N   T  S  L  L
PxGV-K1    GGATCACCAA TCTGGAGACC ACACTCAACA ACTTCGTGAC CACCACCAAC ACGAGCCTGC   660
AF270937   .......... .......... ......G... .......... .......... ..........
             T  L  Q   A  D  I   T  N  I  L   D  L  L   T  P  L   T  Q  A  L
PxGV-K1    TGACCTTACA GGCAGACATT ACAAACATCT TAGATTTGCT AACACCGTTG ACTCAGGCGC   720
AF270937   ..T....... ...G...... .......... .......... .......... ..........
             S  N  T   I  N  T   L  N  P  F   V  A  G   I  V  A   A  T  P  T
PxGV-K1    TATCCAACAC TATCAACACG TTAAATCCTT TTGTGGCCGG AATAGTGGCG GCGACTCCGA   780
AF270937   .......... .......... .......... .......... .......... ..........
             F  D  S   T  T  Y   P  V  V  P   I  V  N   P  L  L   N  P  P  A
PxGV-K1    CCTTCGACTC CACCACATAC CCTGTGGTAC CCATAGTGAA TCCGTTACTC AACCCTCCGG   840
AF270937   .......... .......... .......... .......... .......... ..........
             A  R  L   A  S  E   D  V  V  D   T  L  D   T  L  Q   K  E  V  K
PxGV-K1    CAGCCAGATT GGCCTCGGAG GACGTTGTCG ACACGTTAGA CACGCTGCAG AAGGAGGTTA   900
AF270937   .......... .......... .......... .......... .......... ..........
             R  F  N   D  Y  T   D  T  F  Q   Q  L  L   K  N  V   K  I  Q  S
PxGV-K1    AGCGGTTTAA CGACTACACC GACACGTTTC AACAGCTTTT GAAAAACGTT AAAATTCAAT   960
AF270937   .......... .......... .......... .......C.. .......... ..........
             A  N  *
PxGV-K1    CGGCAAATTA AACGTTAGCT ATTCTCAATA AGATAAATTA ATGGCTAATA TATGCGCAGA  1020
AF270937   .......... .......... .......... .......... .......... ..........

PxGV-K1    TTCTAGA                                                            1027
AF270937   .......
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Fig. 3. Nucleotide sequence of the putative p10 gene, PxORF21, of PxGV-K1. The nucleotide sequence of PxORF21 of PxGV-K1 (upper line) was compared with that of PxGV previously reported by Hashimoto *et al.* (2000). Dots indicate the same nucleotide sequence. The deduced amino acid sequence was indicated with one-letter code designations.

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PxGV-K1     CACAACGTGT ATCTGGACAC ATAACTGGAC AAGTATTTTA CGCACTGCGG AAACGGTTGC    60
AF270937    .......... .......... .......... .......... .......... ..........

PxGV-K1     GATGTTATAG ATAATATATT ACAAAAAGCC ATGTGCGTCG TCGAAAGCAA TCCGACGTTT   120
AF270937    .......... .......... .......... .......... .......... ..........
                                                              M  S   S  I  K
PxGV-K1     AATAATTTTA AAAACAAACA CAATTATACA TAAGAAGATT TACAAATGTC CTCGATTAAA   180
AF270937    .......... .......... .......... .......... .......... ..........
             D  L  Y   N  E  I  I   K  T  Q   Q  D  I   A  V  T  Y   Q  R  V
PxGV-K1     GATTTATACA ACGAGATTAT CAAAACTCAG CAAGACATAG CGGTGACTTA TCAGAGAGTG   240
AF270937    .......... .......... .......... .......... .......... ..........
             A  A  V   E  N  E  I   K  K  K   L  Q  E   H  A  N  S   N  T  V
PxGV-K1     GCAGCGGTAG AAAATGAAAT TAAGAAAAAA TTACAAGAGC ACGCCAATTC AAACACGGTA   300
AF270937    .....A.... .......... .......... .......... .......... ..........
             D  Y  K   L  D  S  V   L  T  Q   L  G  A   T  V  P  L   L  S  K
PxGV-K1     GATTACAAAC TGGACTCTGT GCTGACCCAA TTGGGAGCGA CGGTGCCATT GCTATCAAAA   360
AF270937    .......... .......... .......... .......... .......... ..........
             I  V  D   S  L  K  P   K  L  D   V  P  K   V  D  V  P   K  V  D
PxGV-K1     ATTGTAGATT CTCTAAAACC CAAATTAGAT GTACCCAAAG TCGATGTACC CAAAGTTGAT   420
AF270937    .......... .......... .......... .......... .......... ..........
             D  V  P   K  V  D  D   V  P  K   I  D  V   P  N  V  D   D  V  P
PxGV-K1     GACGTACCCA AAGTTGATGA CGTACCCAAA ATTGATGTAC CCAATGTAGA TGACGTACCC   480
AF270937    .......... .......... .......... .......... .......... ..........
             K  I  D   V  D  D  T   N  V  T   D  E  T   S  N  L  N   E  V  V
PxGV-K1     AAAATCGATG TAGATGACAC CAACGTAACT GACGAAACGT CAAATTTAAA TGAAGTTGTA   540
AF270937    .......... .......... .......... .......... .......... ..........
             V  E  S   P  N  N  H   V  E  T   *
PxGV-K1     GTGGAATCGC CTAACAATCA TGTAGAAACC TAACATCAGT TTAGATATAA ATGTAAACAA   600
AF270937    .......... ....A..... .......... .......... .......... ..........

PxGV-K1     TAAAATTTAT GTTTATTTTT TTTACATACA AAATTTATAA TATTTTTAAA ATTAGAGAAC   660
AF270937    .......... .......... .......... .......... .......... .C..C.....

PxGV-K1     TAAAAAATCG TAAATCGACC C                                              681
AF270937    .......... .......... .
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Fig. 4. Nucleotide sequence of the putative p10 gene, PxORF50, of PxGV-K1. The nucleotide sequence of PxORF50 of PxGV-K1 (upper line) was compared with that of PxGV previously reported by Hashimoto *et al.* (2000). Dots indicate the same nucleotide sequence. The deduced amino acid sequence was indicated with one-letter code designations.

the nucleotide sequences of three putative p10 genes, PxORF2, PxORF21 and PxORF50, were compared, the nucleotide sequence of PxORF21 of PxGV-K1 was different at 7 bases in coding region with that of reported (Fig. 3). Also, the nucleotide sequence of PxGV-K1 PxORF50 showed difference at 4 bases, 1 base in coding region and 3 bases in 3' untranslated region, respectively, with that of previously reported PxORF50 (Fig. 4). These results suggested that the PxGV-K1 isolated in this study is a novel isolate containing different genomic information with previously reported PxGV isolates.

In conclusion, the Korean isolate, PxGV-K1, was determined to be a novel GV isolate through the comparison of viral gene sequences, although it had the general characteristics of PxGV. In particular, our isolation study will increase the knowledge concerning geographical variation in PxGV and may aid in the development of more effective virus strains for biological control of *P. xylostella* and other lepidopteran pests.

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References

Abdul Kadir, H. A., C. C. Payne, N. E. Crook, J. S. Fenlon and D. Winstanley (1999a) Characterization and cross-transmission of baculoviruses infective to the diamondback moth, *Plutella xylostella*, and some other Lepidopterous pests of brassica crops. *Biocontrol Sci. Technol.* **9**, 227-238.

Abdul Kadir, H. A., C. C. Payne, N. E. Crook, J. S. Fenlon and D. Winstanley (1999b) The comparative susceptibility of the diamondback moth, *Plutella xylostella*, and some other major lepidopteran pests of brassica crops, to range of baculoviruses. *Biocontrol Sci. Technol.* **9**, 421-433.

Asayama, T. and N. Osaki (1970) A granulosis of the diamondback moth, *Plutella xylostella. J. Invertebr. Pathol.* **15**, 284-286.

Hamm, J. J. and J. D. Paschke (1963) On the pathology of a granulosis virus of the cabbage looper, *Trichoplusia ni* (Hübner). *J. Insect Pathol.* **5**, 187-197.

Huger, A. (1963) Granuloses of insects; in *Insect Pathology: An Advanced Treatise Vol. 1.* Steinhaus, E. A. (ed.), pp. 531-575, New York, America Press.

Kao, H. W. and R. I. Rose (1976) Effect of sunlight on the virulence of the granulosis virus of the diamondback moth and evaluation of some protective adjuvants. *Plant Protection Bulletin (Taiwan)* **18**, 391-396.

Laemmli, U. K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Rohrmann, G. R. (1992) Baculovirus structural proteins. *J. Gen. Virol.* **73**, 749-761.

Shim, H. J., J. Y. Roh, J. Y. Choi, M. S. Li, S. D. Woo, H. W. Oh, K. S. Boo and Y. H. Je (2003) Isolation and characterization of a *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus isolate in Korea. *J. Microbiol.* **10**, 306-311.

Tanada, Y. and H. K. Kaya (1993) Insect Pathology. Academic Press, San Diego.

Tarashnik, B. E., Y-B. Liu, T. Malvar, D. G. Heckel, L. Masson, V. Ballester, F. Granero, J. L. Mensua and J. Ferre (1997) Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis. Proc. Natl. Acad. Sci. USA* **94**, 12780-12785.

Tweeten, K. A., L. A. Bulla and R. A. Consigli (1981) Applied and molecular aspects of insect granulosis viruses. *Microbiol. Rev.* **45**, 379-408.

Wang, C. L. and R. I. Rose (1978) Control of the imported cabbageworm, *Pieris rapae crucivora* Boisduval, with granulosis virus in the field. *Plant Protection Bulletin (Taiwan)* **20**, 16-20.

Yoshifumi, H., H. Tohru, U. Yasumass, F. Tomonori, S. Yoshitaka and M. Tsuguo (2000) Sequence analysis of the *Plutella* *xylostella* granulovirus genome. *J. Virol.* **275**, 358-372.

Zhao, J. Z., H. L. Collins, J. D. Tang, J. Cao, E. D. Earle, R. T. Roush, S. Herrero, B. Escriche, J. Ferre and A. Shelton (2000) Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. *Appl. Environ. Microbiol.* **66**, 3784-3789.

Zhang, X. X., Z. P. Liang, H. Y. Peng, Z. X. Zhang, X. C. Tang and T. Q. Liu (2005) Characterization and partial genome sequence analysis of *Clostera anachoreta* granulovirus. *Virus Res.* **113**, 36-43.