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Isolation and Characterization of a *Lymantria dispar* Multinucleocapsid Nucleopolyhedrovirus Isolate in Korea

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In Korea, a *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus, LdMNPV-NM, was isolated and characterized from dead *L. dispar* larvae. The polyhedra of LdMNPV-NM were irregularly shaped with a diameter of 1.62±0.33 μm. Numerous virions comprised of the multinucleocapsid were evident in the electron microscopic examination of the polyhedra cross sections. These polyhedra were composed of a major protein of 30 kDa. The restriction enzyme digestion patterns of LdMNPV-NM showed that this isolate had some different fragments from those of the Gypchek® LdMNPV isolate, although their overall profiles were similar. The deduced amino acid sequence of the *enhancin* gene of LdMNPV-NM showed differences when compared to previously reported *enhancin* genes of other LdMNPV strains. These results suggested that the LdMNPV-NM isolate from Korea was a new NPV strain and had a new *enhancin* gene.

Key words: baculovirus, LdMNPV-NM, enhancin, *Lymantria dispar*

Nucleopolyhedrovirus(NPV) is a member of the *Baculoviridae* which infect insects and other arthropods. *Lymantria dispar* multinucleocapsid nucleopolyhedrovirus (LdMNPV) is known to be pathogenic to the gypsy moth, *L. dispar*, a serious pest of forest fruit and shade trees throughout the world. LdMNPV has contributed to the decline of gypsy moth populations, and several strains of LdMNPV have been reported in the United States, Europe and Asia (Shapiro *et al.*, 1984; Narang *et al.*, 2001).

LdMNPV has a genome size greater than 160 kb which is significantly larger than the three other sequenced baculovirus genomes: *Autographa californica* MNPV (AcMNPV), *Bombyx mori* SNPV (BmSNPV), and *Orgyia pseudotsugata* MNPV (OpMNPV) genomes (Reigel *et al.*, 1994). The extra 29 kb of DNA present in the LdMNPV suggests that it has the potential to possess several genes which are not present in AcMNPV. Several genes encoding proteins that are only present in LdMNPV genome, such as host range factor 1 (Thiem *et al.*,1996), enhancin (Bischoff and Slavicek, 1997) and ribonucleotide reductase, etc., have been identified and characterized.

Enhancins, which have been referred to as the synergistic or viral enhancing factor, are a group of proteins first identified in the granuloviruses (GV) occlusion body that have the ability to enhance the infection of some NPVs (Tanada and Hukuhara, 1971; Gallo *et al.*, 1991). *Trichoplusia ni* GV (TnGV) enhancin demonstrated that the major effect of enhancin appears to be an increase in infection efficiency due to the insect peritrophic membrane degradation (Gallo *et al.*, 1991). This enhancin was later found to be a metalloprotease, which degrades mucin that is a major protein constituent of the peritrophic membrane (Lepore *et al.*, 1996; Wang and Granados, 1997). Recently, Hayakawa *et al.* (2000) performed bioassays to examine the enhancement of the baculovirus infection of *Spodoptera exigua* larvae by using a recombinant baculovirus and tobacco plant engineered with the TnGV *enhancin* gene. The first GV homolog found in LdMNPV was the *enhancin 1* (E1) gene, which was also the first *enhancin* gene found in the NPVs (Bischoff and Slavicek, 1997). A second *enhancin* gene (E2) was recently identified in a LdMNPV when the entire genome of isolate CI 5-6 was sequenced (Kuzio *et al.*, 1999). These two enhancin genes contributed to the viral potency of wild-type LdMNPV (Popham *et al.*, 2001).

In the present study, the new isolate, LdMNPV-NM was characterized as a potential source of new NPV strains and its new *enhancin* gene was sequenced and compared to previously reported *enhancin* genes.

Materials and Methods

Viral isolates

The virus-infected *L. dispar* cadavers were collected from

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Gyeonggi province, Korea, and they were homogenized by vortexing with distilled water and strained through four layers of cheesecloth. The mixture was centrifuged at 5,000 rpm for 15 min, and then, the pellet was washed three times with distilled water. The viral occlusion bodies (OBs) were layered onto 40%-65% (w/v) discontinuous sucrose gradient and were centrifuged at 80,000×g for 60 min. The OBs were collected and pelleted by centrifugation at 10,000×g for 10 min. Then, they were resuspended with 0.5 M NaCl (Stiles *et al.*, 1983). The purity of the isolate was confirmed by the restriction endonuclease analysis, and no submolar DNA fragments were detected (Fig. 3). Gypchek® LdMNPV isolate was provided from the entomology department of the University of Massachusetts and was prepared by same method as above.

Insect rearing

L. dispar egg masses were collected in Gyeonggi province, Korea and were maintained in a growth chamber at 25±2°C,60% RH, and a 16 L:8 D photoperiod. After hatching, the larvae were reared on a high wheat germ diet (Bio-Serv®, USA).

Electron microscopy

For scanning electron microscopy, the purified OBs were fixed in a 2.5% paraformaldehyde-glutaraldehyde mixture buffered with 0.1 M phosphate (pH 7.2) for 2 h, post-fixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in graded ethanol, and substituted by isoamyl acetate. Then they 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 scanning electron microscope (SEM 515, Philips, Netherlands). The diameter of each OB was measured.

For transmission electron microscopy, the post-fixed samples were dehydrated in graded ethanol and propylene oxide, and were embedded in Epon-812. Ultra-thin sections, made by ULTRACUT E (Leica, Germany) ultramicrotome, were stained with uranyl acetate and lead citrate, and these sections were examined under CM 20 (Philips, Netherlands) electron microscope.

SDS-PAGE

The OBs were mixed with an equal amount of the 2× sample buffer (5% SDS, 10% β-mercaptoethanol, 0.02% bromophenol blue, 20% glycerol). The samples were boiled for 5 min and were clarified by centrifugation (10,000×g for 1 min). The total cellular lysates were performed on a 12% polyacrylamide separating gel with a 3% stacking gel containing SDS as described by Laemmli (1970). The gel was stained with Coomassie brilliant blue. Molecular weight marker (Sigma, USA) was used as standard.

Restriction endonuclease digestion analysis

Viral DNA was extracted according to the procedure described by O'Reily *et al.* (1992) and was digested with four restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII and *Nco*I) according to the manufacturer's manual. The samples were analyzed by electrophoresis on a 0.7% agarose gel and were stained with 0.5 μg/ml ethidium bromide.

Bioassay

The OBs were quantified by visual counting in a hemacytometer. They were diluted in distilled water to produce concentrations ranging from 10^2 to 10^6 OBs/ml and 200 μl of each dilution was added to each diet (2×3×1 cm^3). Bioassays were replicated three times with 20 second instar larvae of *L. dispar* per treatment. The larvae were reared in a growth chamber at a constant temperature of 25±2°C and were observed from day 1 to day 14. Dead larvae were counted and dose-mortality data was determined by Probit analysis using a POLO-PC program (LeOra Software, USA).

Cloning and sequence analysis of the enhancin gene

Viral DNA was digested with *Sph*I, *Sac*II and *Sac*II/*Sph*I and was fractionated on 0.7% agarose gels. Southern blot analysis was performed on a nylon membrane with the nonradioactive DNA labeling and detection kit (Boehringer Mannhein, Germany). The 1.1 kb PCR product containing the partial *enhancin* gene (amplificated with primers referred to *enhancin* gene, Genbank No. NC 001973, CDS 61463-62619) was used as a probe.

The DNA sequence of the *enhancin* gene was determined on an ABI sequencer Model 377 (ABI system, USA). The obtained sequence was compared with the known *enhancin* genes from other LdMNPVs and TnGV using the BLAST and DNASTAR Program.

Results and Discussion

Characterization of LdMNPV-NM

The polyhedra of the LdMNPV-NM isolate were observed as irregularly shaped, proteinaceous occlusion bodies (Fig. 1A), which are general features of LdMNPV (Steinhous, 1949). The average diameter of the polyhedra was 1.62± 0.33 μm. Transmission electron microscopy revealed that LdMNPV-NM had bundles of virions in the nucleocapsid, which belonged to MNPV (Fig. 1B). To identify the polyhedral protein of LdMNPV-NM, SDS-PAGE was performed (Fig. 2). Polyhedrin protein was determined to have a MW of 30 kDa, which was similar to those of the Gypchek® LdMNPV and BmNPV (30 kDa) but different from that of the AcMNPV (31 kDa), and these results were in agreement with a previous report (Stiles *et al.*, 1983). The viral DNAs of LdMNPV-NM and Gypchek® LdMNPV were analyzed by comparing the fragmentation profiles that resulted from their digestion with *Bam*HI, *Eco*RI, *Hin*dIII and *Nco*I. Their viral DNA profiles showed a high similarity between the two types. However, there were distinct differ-

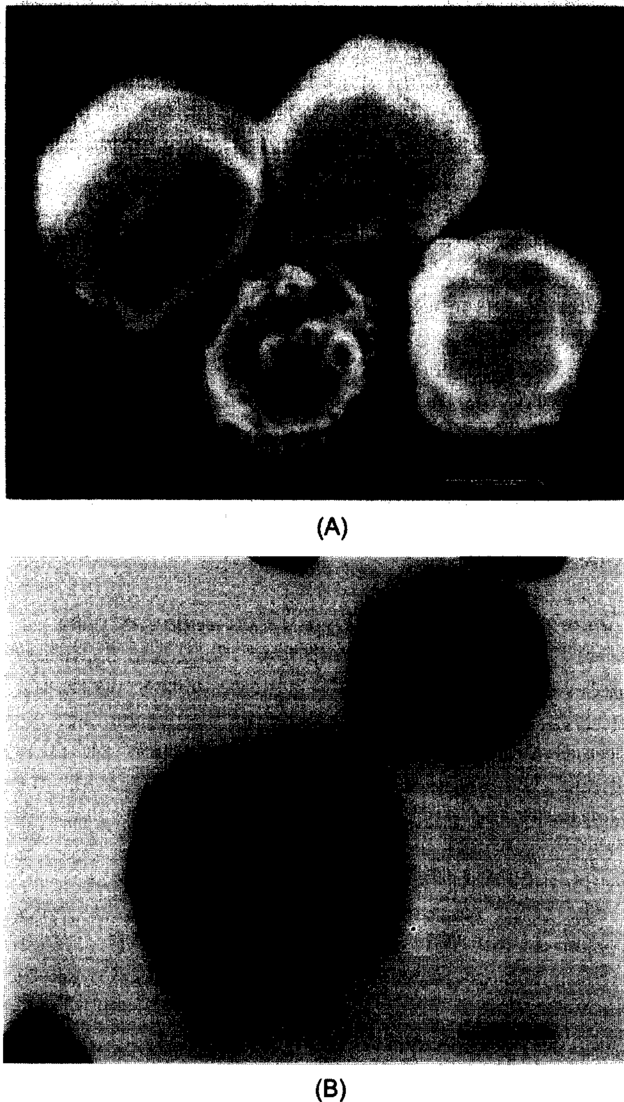

Fig. 1. Scanning electron (A) and transmission electron (B) micrograph of polyhedra of LdMNPV-NM. Scale bar indicates 1 μm.

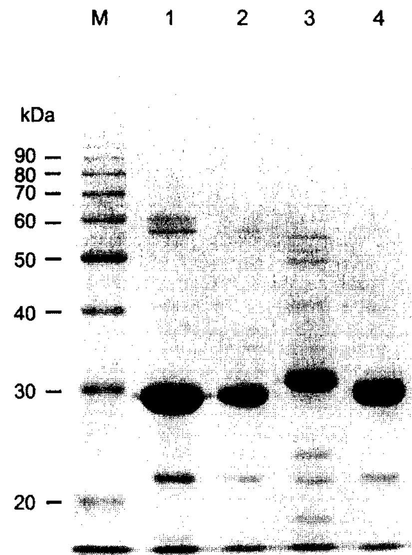

Fig. 2. SDS-polyacrylamide gel electrophoresis of the polyhedrin of LdMNPV-NM. Lanes: M, protein molecular weight marker; 1, LdM-NPV-NM; 2, Gypchek® LdMNPV; 3, AcMNPV; 4, BmNPV.

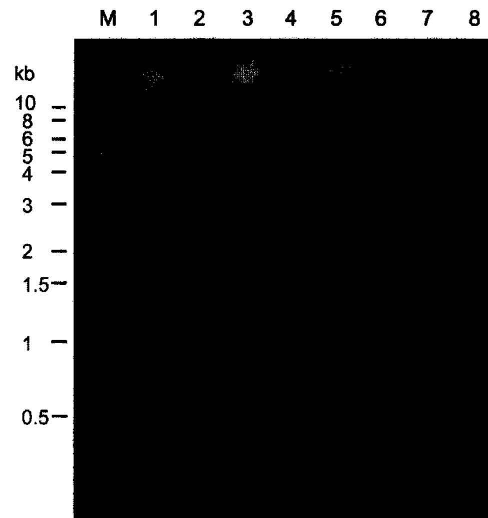

Fig. 3. Restriction endonuclease digestion profiles of genomic DNAs of LdMNPV-NM and Gypchek® LdMNPV. Viral DNAs of LdMNPV-NM (lanes 1, 2, 3 and 4) and Gypchek® LdMNPV (lanes 5, 6, 7 and 8) were digested with the restriction endonuclease *Bam*HI (lanes 1 and 5), *Eco*RI (lanes 2 and 6), *Hin*dIII (lanes 3 and 7) and *Nco*I (lanes 4 and 8).

ences in some of the digested fragments, such as a *Bam*HI 3 kb fragment of LdMNPV-NM (Fig. 3). The common viral DNA profiles between the two strains were in agreement with a previous report (Narang *et al.*, 2001). Although they are of the same virus species, this kind of heterogeneity is due to different geographical origins. Furthermore, these results proved that baculovirus genomes could undergo deletion, point mutation, recombination, etc (Murillo *et al.*, 2001).

A previous study on comparative infections of LdMNPV strains from North America, Europe, and Asia against neonate larvae showed that LC_{50} values varied from 1.7×10^3 OBs/ml to 5×10^6 OBs/ml (Shapiro *et al.*, 1984). Slavicek *et al.* (1992) reported that LdMNPV A2-1, a different isolate from Gypchek®, had a LC_{50} value of 9.90×10^3 OBs/ml for the newly molted second instar *L. dispar* larvae. In this study, the LC_{50} of LdMNPV-NM was 4.99×10^4 OBs/ml, and it was three-fold higher than that of Gypchek® LdM-NPV (Table 1). These differences in biological activity, as

Table 1. Lethal concentration of LdMNPV-NM and Gypchek® LdM-NPV for the second instar *L. dispar* larvae

Isolate	LC_{50} (95% FL)[1]	LC_{90} (95% FL)	Slope
NM	4.99 (2.40-9.42)	64.31 (28.40-295.88)	1.16±0.22
Gypchek®	1.92 (0.71-3.66)	22.76 (10.27-128.19)	1.19±0.28

[1]Polyhedra/ml infection sol. $\times10^4$; FL, fiducial limits.

well as differences in host susceptibility, can be detected among the geographical "races" of the same NPV species. Vasiljevic and Injac (1973) studied susceptibilities of *L. dispar* larvae from different areas, such as Yugoslavia, Bulgaria and the USSR, to LdMNPV also from different regions. Larvae from Bulgaria were most susceptible to isolates from the USSR and the United States and were least susceptible to isolates from Croatia and Macedonia. Larvae from Yugoslavia were most susceptible to LdMNPV from Japan and the United States and were least susceptible to LdMNPV

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LdMNPV-NM       MSNTITFTLTVLPTTLPVFLSASDTSLGLHHNRVPLGYVCSSATTITVRS-------TAAISLRFLNNNRNQERSVTVEANSSSSF-THNQTYVPFADRVV    93
LdMNPV CI5-6    MSNTTTFTLTVLPTTLPVFLSASDTSLGLHHNRVPLGYVCSSATTITVRS-------TAAISLRFLNNNRNLERSVTVEANSSSSF-THNETYVPFADRVV    93
LdMNPV A21-MPV  MSNTTTFTLTVLPTTLPVFLSASDTSLGLHHNRVPLGYVCSSATTITVRS-------TAAISLRFLNNNRNLERSVTVEANSSSSF-THNETYVPFADRVV    93
TnGV            MS----YKVIVPATVLPPWLRVGENWIFARHRRTEVGVVLPANTKFRVRADFSRAGFTRPVIVRLLNNNRSTERE INLNNDQWMEV-EHAHESVPFVDWLV    96
LdMNPV E2       ME----YALSIAPTRVPQWLAPDTNQLGLHHGRVHFNCVCGASSGVRIR--MRNAARVPRLTLRFLNADSREERTVVVDASNDDTFWSHDRLAVPFVDRPV    95

LdMNPV-NM       GGDARGYVVECTVNNYLSVLPHYTHG-LTDEAAFKNELRALDDNGSFAFLELKNALLLVPPPDKAELLA--LDLGALDNFYTTIVDTFDHLIGLVNVASDD   191
LdMNPV CI5-6    GGDARGYVVECTVNNYLSVLPHYTHG-LTDEAAFKNELRALDDNGSFAFLELKNALLLVPPPDKAELLA--LDLGALDNFYTTIVDTFDHLIGLVNVASDD   191
LdMNPV A21-MPV  GGDARGYVVECTVNNYLSVLPHYTHG-LTDEAAFKNELRALDDNGSFAFLELKNALLLVPPPDKAELLA--LDLGALDNFYTTIVDTFDHLIGLVNVASDD   191
TnGV            GEKNTMAEVYFEIDGPHIPLPVYVFN-TRPVEHPKSEYR--QSSSGYCFLYLDLVCMLVPPASKNALLD--VNIFELHQFYNEIINYYDDLCGLVEDPYAD   192
LdMNPV E2       GDEPADVSVTITNPAY--ALPLYEHGGNVAPADFKRSWR--DSVSPYALVDIGNTAFLVPPADKELVTADAFDLIRLRDHYESIYRHYENAIVVSDSPTAP   192

LdMNPV-NM       PATRNF-NKKYFCKADSN-GAGAAYYDRNNTAQTNVSNTRYLQPRATNNLVL[illegible]AYDFGFAGNTPALNEVNNNVLADRYQYDFNSFDERGRDASVYEN   290
LdMNPV CI5-6    PATRNF-NKKYFCKADSN-GVGAAYYDRNNTAQTNVSNSRYLQPRKTNNLVL[illegible]AYDFGFVSNTPALNEVNNNVLADRYQYDFNSFDERGRDASVYEN   290
LdMNPV A21-MPV  PATRNF-NKKYFCKADSN-GVGAAYYDRNNTAQTNVSNSRYLQPRATNNLVL[illegible]AYDFGFVSNTPALNEVNNNVLADRYQYDFNSFDERGRDASVYEN   290
TnGV            TVDSNLPNKAAFVKADAG-GPGGAYYGPFWTAPASSNLGDYLRISPTNWMVI[illegible]L[illegible]AYDFVFTVNT-ILIEIWNNSLCDRIQYKWWNKIKRQQLARVYEN   290
LdMNPV E2       AADRVYA-RQFFCKADAGSGAGIAFYGQHWIGASANTLLRYLNVDADPWLIL[illegible]GHEFAFVGTAPPLIEVNTNILPNLFQFDTMTSAERQTAAWIYDH   292

LdMNPV-NM       GNRDRVERNIAERIDNRAPFDSWSFFQKMAVFTWMMDTDCGRETMARINRQFRQIKTFDSSPRYMPTFDWNVLLSDGDFVPYLKLMQVEFTSCRVLSNNNV   391
LdMNPV CI5-6    GNRDRVERNIAERIDNRAPYESWSFFQKMAVFTWMMDTDCGRETMARINRQFRQIKTFDSSPRYMPTFDWNVLLSDGDFVPYLKLMQVEFTSCRVLSNNNV   391
LdMNPV A21-MPV  GNRDRVERNIAERIDNRAPYESWSFFQKMAVFTWMMDTDCGRETMARINRQFRQIKTFDSSPRYMPTFDWNVLLSDGDFVPYLKLMQVEFTSCRVLSNNNV   391
TnGV            -RRPQKEATIQALIDNNSPFDNWGFFERLIIFTWLYNPQRGLDTLRNINHSYRVHATRNSSIPYPQIWSWLTTSAYDNFWLYFNLVGVYPADFYVNEHNKV   391
LdMNPV E2       GRREVVERGLGALIDQRVDYDKFSFRERLFAYAPLTQTSAGRRAFQRMHLQLRRFNMRGDGPTEYLIADWWSNAAEMDCLPLFMLMQQKVVSCCRYGRD-V   392

LdMNPV-NM       VDTFLHTNSLVRSKRVYYPVKELIANFDALTNNYGFVA--QSNYSLVAPGEVDARASLV---IHCDIDDARQIAGQIFYVYDGTRLVWQSQIDNSNRLVVN   487
LdMNPV CI5-6    VDTFLHTNSLVRSKRVYYPVKELIANFDALANNYGFVA--QSNYSLVAPGEVDARASLV---IHCDIDDARQIAGQFFYVYDGTRLVWQSQIDNSNRLVVN   487
LdMNPV A21-MPV  VDTFLHTNSLVRSKRVYYPVKELIANFDALANNYGFVA--QSNYSLVAPGEVDARASLV---IHCDIDDARQIAGQFFYVYDGTRLVWQSQIDNSNRLVVN   487
TnGV            VHFNLHLRALALGQSVRYPIKYIITDFDLVSKNYDIKQYLESNFDLVIPEELRQTDLLADVRVVCVIDDPSQIVGEPFSVYDGNERVFESTVATDGNMYLV   492
LdMNPV E2       RPVYTFERALSAFKRVPYPACRLLSDFDTQSNNYGMPG-LETTFAPVYPAQSDAS--VAELTIVMHIDEPAQIAGEPVELFDGSRLVCAAAVDGARARFGS   490

LdMNPV-NM       DIHAGVYTMVAPRGRDKRYRVYFDEPA--SIAGLNEHLYLFADTSKPTKRLIYERLDSSPAGDRVAAHVLGINDL-YRAKVIFDFKAKLMSVHSFASSFNS   585
LdMNPV CI5-6    DIHAGVYTMVAPRGRDKRYRVYFDEPA--SIAGLNEHLYLFADTSKPTKRLIYERLDSSPAGERVAAHVLGINDL-YRAKVIFDFKAKLMSVHSFASTFNS   585
LdMNPV A21-MPV  DIHAGVYTMVAPRAH-KRYRVYFDEPA--SIAGLNEHLYLFADTSKPTKRLIYERLDSSPAGDRVAAHVLGINDL-YRAKVIFDFKAKLMSVHSFASTFNS   584
TnGV            GVGPGVYTLRAPRGKNKRYKLHLAHSPREPVHPANDHMYLLVTYPYYNQTLTYTPYVNSDLAVDMA-HLFGSNDRRYVATIYFNPFEQTVTVHLNNIRAGR   592
LdMNPV E2       HVPVGVYGLHVPRGRDRRYDITIPDNRFNDKGNGCTNLYAIVTNTTARVDVLYEPKRAPSVIARPAGYLMGYSD-RLAAGFVVDVAKRRVELTVYRPQLHW   590

LdMNPV-NM       GYANMRYFVINVKQDQLYTFNHTFTGTQNFVGVM----SVDIAPGDTMSSFHQQGGT----RFIFLNA--KSLNFTLNVSENWYSNTNLPSKNQVLE----   672
LdMNPV CI5-6    GYANMRYFVLNIRRDQLYTFNHTFTGTQNFVGVM----SVDIAPGDTMSSFHQQGDT----RFIFLNA--KSLNFTLNVSENWYSNTNLPSKNQVLE----   672
LdMNPV A21-MPV  GYANMRYFVLNIRRDQLYTFNHTFTGTQNFVGVM----SVDIAPGDTMSSFHQQGDT----RFIFLNA--KSLNFTLNVSENWYSNTNLPSKNQVLE----   671
TnGV            ENNTTLYFEMVIS-NPFNGQSQTFTILEDNPTLRQGYYKFDVVTYSSIRLNMSVAGRLLFRRYIFAGG--TT---TLTMFPNQVLEPNLFPDGSALNRTL-   686
LdMNPV E2       FF- VQYFTIAVHRNGALVGELVALGNHGDADYHQ---SVEYQPNDVMTLTLLDNATR--DRLIFMGAALQQLQTQYLLAANGVQDLSDQPELASAERQIR   684

LdMNPV-NM       TRMDECVAYLYANASRLIPFENHLKDELYLTIQSL-PDKDYYMRLYNPFLPAH------FKFNTFD----------PTTGSSSIMMA-------------   742
LdMNPV CI5-6    TRMDECVAYLYANASRLIAFENHLKDELYLTIQSL-PDKDYYMRLYNPFLPAH------FKFNTFD----------PTTGSSSIMMA-------------   742
LdMNPV A21-MPV  TRMDECVAYLYANASRLIAFENHLKDELYLTIQSL-PDKDYYMRLYNPFLPAH------FKFNTFD----------PTTGSSSIMMA-------------   741
TnGV            ARLREQAAFL-DNYSQLMYIENELRDTIYLASQLVDPASDEFVKYYPDYFRDPHTYVYLFRFRGLGDFVLLDLQIVPLLNLATVRIANIQNGPHSYFDTLY   786
LdMNPV E2       TKLSEVTGFL-DSEPSLLRIENWLKDDILLMIQAL-PDSQALMRTHCRYLPKHFKCVESAPYAPW- WSVLSLVAV---GVVCLLL------------LF   765

LdMNPV-NM       ----------VVIFCFV------LVALVIVF-------ILVFVNRRGRQ----SPKAAERAPPPLQRV----------------------------------   783
LdMNPV CI5-6    ----------VVIFCFV------LVALVIVF-------ILVFVNRRGRQ----SPKAAERAPPPLQRV----------------------------------   783
LdMNPV A21-MPV  ----------VVIFCFV------LVALVIVF-------ILVFVNRRGRQ----SPKAAERAPPPLQRV----------------------------------   782
TnGV            FKVELRDTNGAIVFSYSRRGNEPMTPEHHKFEVVYSGYTVELFMREPQNRLQLIVNKMLDTALPSTQNIFARITDTQLVVGDTSIEDNLVTSINVDCGDDDN   887
LdMNPV E2       F-------------------------------------VIATIARRAKR---------DDARPP---------------------------------------   784

LdMNPV-NM       --------------   783
LdMNPV CI5-6    --------------   783
LdMNPV A21-MPV  --------------   782
TnGV            QKIRVVETLKMIAF   901
LdMNPV E2       ---------SVIKA   789
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Fig. 4. Amino acid alignment of the LdMNPV-NM enhancin protein with those of LdMNPV CI 5-6, A21-MPV, TnGV and enhancin 2 (E2) with the DNASTAR program. Shaded boxes indicate identical residues conserved in the five enhancin proteins. Identical residues within the conserved zinc-binding domain of all metalloproteases are indicated by the black boxes.

from Slovenia and Macedonia. This result suggested that pathogenicity of LdMNPV-NM was not significantly different from those of known LdMNPV isolates although LC_{50} value of LdMNPV-NM was relatively high.

Characterization of the enhancin gene

In the Southern hybridization analysis of LdMNPV-NM, the *enhancin* gene probe hybridized strongly with 4.5 kb *Sac*II fragment and 3.3 kb *Sac*II/*Sph*I fragment DNA of LdM-NPV-NM, where both fragment lengths are approximate (data not shown). The 3.3 kb *Sac*II/*Sph*I fragment containing the *enhancin* gene was cloned into pGEM-5Zf(−) to generate pGEM-LEnh, and its nucleotide sequence was analyzed. The *enhancin* open reading frame (ORF) of LdMNPV-NM was composed of 2,352 nucleotides and encoded a 783-amino acid protein with a predicted molecular mass of approximately 89 kDa. Analysis of the sequence upstream of the *enhancin* ORF revealed a potential baculovirus late promoter sequence, TTAAG, which begins 13 bp upstream of the enhancin start codon. Immediately downstream of the LdMNPV-NM *enhancin* gene was the *hrf-1* gene, and there was no potential polyadenylation signal sequence between the *enhancin* stop codon and *hrf-1* start codon (data not shown).

The deduced amino acid sequence of the cloned *enhancin* gene was compared to the amino acid sequences of previously reported *enhancin* genes of LdMNPV and TnGV (Fig. 4). The LdMNPV-NM enhancin exhibited amino acids identities of about 97.5, 97.3, 31.7 and 27.2% with those of the two LdMNPV isolates (CI 5-6 and A21-MPV), and TnGV, and with enhancin 2 (E2) of LdMNPV, respectively (Bischoff and Slavicek, 1997; Kuzio *et al.*, 1999; Popham *et al.*, 2001). The predicted enhancin protein of LdMNPV-NM also contained a conserved zinc-binding domain characteristic of metalloproteases. The signature pattern, HEXXH, is sufficient to group a protein into the metalloprotease superfamily. All five enhancin proteins had this conserved metalloprotease zinc-binding domain (Fig. 4). It has been demonstrated that the TnGV *enhancin* gene encodes a metalloprotease that is capable of degrading an insect intestinal mucin in the *T. ni* larval midgut. The contribution of LdMNPV *enhancin* genes to the virulence of LdMNPV has been documented (Bischoff and Slavicek, 1997; Popham *et al.*, 2001); however, whether an *enhancin* from LdMNPV can enhance the virulence of a heterologous NPV is not clear. In further studies, we will report the analysis of synergistic function of LdMNPV-NM *enhancin* by its expression using the baculovirus expression system.

In conclusion, the Korean isolate, LdMNPV-NM, was determined to be a new NPV isolate through the comparison of viral DNA profiles and *enhancin* gene with known isolates as well as Gypchek® LdMNPV, although it had the general characteristics of LdMNPVs. In particular, our isolation study will increase the knowledge concerning geographical variation in LdMNPVs and may aid in the development of more effective virus strains for biological control of gypsy moths and other pests. In addition, further study of the other Korean strains is needed in terms of the selection of active isolates of LdMNPV having most pathogenicity in Korea.

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