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A Cytoplasmic Polyhedrosis Virus Isolated from the Oriental Tobacco Budworm, *Heliothis assulta* Guenée (Lepidoptera: Noctuidae)

담배나방 세포질다각체병 바이러스의 동정 및 병원성에 관한 연구

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ABSTRACT A cytoplasmic polyhedrosis virus isolated from the oriental tobacco budworm, *Heliothis assulta* (HaCPV), was studied on morphology of the polyhedron and virus particles, analysis of viral protein and nucleic acid, and bioassay of the HaCPV to determine the feasibility of application as a microbial control agent. The shape of polyhedron was hexagonal ranging 0.5-3.7 μm and the virus particles were icosahedral outline measured 55 nm in diameter. Polyhedral protein was composed of a major polypetide of 24.3 Kd and 5 minor components and virus particle had seven polypeptides ranging in 28.0 Kd-133. 6 Kd by the SDS-PAGE. The genome of virus was segmented with 10 double stranded RNA in the total mol. wt. of 18.08 Md ranging in 0.65 Md -2.79 Md. The LC_{50} values of the HaCPV to the 3rd instar of *H. assulta* larvae were calculated to 2.895×10^5PIBs/ml. The LT_{50} values in the concentration of 5.0×10^6PIBs/ml was 16.4 days.

KEY WORDS *Heliothis assulta*, cytoplasmic polyhedrosis virus, viral protein, genome, bioassay

초 록 담배나방 유충에서 분리한 세포질다각체병 바이러스의 형태, 다각체 단백질 및 핵산의 전기영동상과 바이러스의 병원성을 조사하여 본 바이러스를 이용한 담배나방의 생물적 방제 이용성을 검토하고자 본 실험을 수행하였다. 다각체의 형태는 외관상 6각형으로 0.5~3.7 μm 크기이고 바이러스 입자는 정 20면체로 55 nm였다. SDS-PAGE에 의한 다각체 단백질은 단일 폴리펩타이드인 24.3 Kd와 5개의 작은 구성분으로 이루어졌다. 바이러스입자는 7개의 폴리펩타이드로 구성되어 있으며 분자량은 28.0~133.6 Kd였다. 바이러스 게놈은 10개의 조각으로 된 총 분자량 18.08 Md인 이본쇄 RNA로 각 조각의 분자량 범위는 0.65~2.79 Md 이였다. 3령 유충에 대한 담배나방 세포질 다각체병바이러스의 LC_{50}은 2.895×10^5PIBs/ml이었으며 5.0×10^6PIBs/ml의 농도에서 LT_{50}에서 16.4일이었다.

검 색 어 담배나방, 세포질 다각체병바이러스, 바이러스단백질, 게놈, 생물검정

Host insects of cytoplasmic polyhedrosis viruses (CPVs) have been recorded in 173 Lepidoptera (Martignoni Iwai 198 6). Recently, van Regenmortal (1990) proposed the genus Cypovirus, the family Reoviridae which is called cytoplasmic polyhedrosis viruses in invertebrates. The CPVs are characterized by the formation of large proteinaceous inclusion bodies within the cytoplasm of the mid-gut epithelial

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cells. Virus particles are icosahedral and have a diameter of 50 to 65 nm and a spike at vertice of the particles (Hosaka & Aizawa 1964). The protein of the polyhedra comprises a major polypeptide of molecular weight from 25 Kd to 30 Kd. The genomes of these viruses are composed of double-stranded RNA and segmented into 10 discrete units ranging in size from 0.6 Md to 3.53 Md with the total mol. wt. of 20.4 Md(Matthews 1982). The classification in the CPVs of insects has provised on the based of the size of the RNA segments and the CPVs were recorded 12 distinct virus types from the insects (Payne & Rivers 1976, Payneet al. 1977). Some CPVs in Lepidoptera have been used for the control of forest insect pests (Katagiri 1981). *Vanessa cardui*, a forest insect pest, was found to be susceptible to 11 species of insects among 18 species of lepidoptera and 2 species of hymenoptera (Neilson 1964). Thus the CPVs have a cross transmission among other insect pests. A CPV isolated from the oriental tobacco budworm, *Heliothis assulta*, which is the first record in the host insect was studied on morphology, analysis of viral protein and nucleic acid and bioassay of *H. assulta* CPV to diagnosis the disease and to test the pathogenicity of the HaCPV to *H. assulta* larvae.

MATERIALS AND METHODS

Virus isolate and production

A cytoplasmic polyhedrosis virus of *H. assulta* (HaCPV) was isolated from the last instar larvae which have been reared on semi-synthetic diet in the insectary, Entomology Division, ASI. Third instar larvae were fed on diet sprayed 10^8 PIBs/ml of the HaCPV on the surface of diet and excised mid-gut tissues at 10 days post inoculation for virus production.

Purification of polyhedra and virus particles

Infected tissues were macerated in a Teflon homogenizer containing 0.01% sodium dodecyl sulfate (SDS) in 0.1M TE buffer and centrifuged twice at 3,000 rpm for 10 min. The pellets suspended in 0.01% SDS were layered on 40 to 60%(w/v) linear sucrose gradient and spun at 20,000 rpm for 30 min in the high speed centrifuge, Hitachi, SRP 28SA. The polyhedral band was washed twice with distilled water by centrifugation at 3,000 rpm for 10 min to eliminate sucrose. The polyhedra suspended in the alkaline solution (0.1M Na_2CO_3 0.01M EDTA, and 0.17M NaCl, pH10.9) were gently shaked for 30 min at 37℃ in a shaker and spinned at 3,000 rpm for 10min to discard the undissolved polyhedra. The supernatant of the suspension was layered on 10% to 50%(w/v) linear sucross gradient and spinned at 25,000 rpm for 1hr. Several bands layered on the middle of the tube were collected with a capillary pipet, washed in 0.1M TE buffer and centrifuged at 20,000 rpm for 30min. The pellets of virus particles were diluted with 0.1M TE buffer(pH 7.5) and washed with the same buffer. Phase contrast microscope and the scanning electron microscope, Hitachi S-570 were used to observe the virus.

Preparation of polyhedral protein and viral RNA

Polyhedral protein was obtained as the above method used in the purification of virus particles except the process of inactivation of any endogenous protease activity in 0.05M TE buffer containing 1.0% SDS, 0.5% β-mercaptoethanol, pH 7.2 and incubated in a 70℃ of water bath for 90min. The polyhedral protein in supernatant fluid were centrifuged at 50,

000 rpm for 30min and added Laemmli's solution (Laemmli 1970). The protein was dissociated by heating at 100℃ for 10 min for polyacrylamide gelelectrophoresis. Viral RNA was extracted from purified polyhedra of virus particles using a phenol-chloroform extracting method (Maniatis 1982). The viral RNA was suspended in TE buffer by the weight of 100 μg of RNA per ml of buffer.

Polyacryamide gel electrophoresis

Polyhedral protein was electrophoresed in a 12.5% SDS-polyacrylamide gel. The gels were stained for 2 hrs in 0.1% Coomassie brilliant blue diluted with acetic acid : methanol : distilled water=10 : 40 : 50, and destained with 0.5 % BFB. 50% glycerol, 0.1M EDTA of stop buffer. Viral RNA was electrophoresed in 3% polyacrylamide gel in 0.1M Tris-HCl containing 2.83% acryamide. 0.17% bis-acrylamide, 0.15% ammonium sulfate, 0.06% TEMED, 0.02M sodium acetate, 0.1% SDS, and 0.001M EDTA, pH7.6 at 25 volt for 14 hrs. The gel was stained with ethidium bromide(0.5 μg/ml) for 1hour and scanned at 302nm after destaining with distilled water.

Bioassay

Third instar larvae of *H. assulta* starved for 12 hours at 10℃ were fed on the surface of 1gm of diet sprayed with 50 μl of the diluted viral suspension from 5.0×10^4 to 5.0×10^8PIBs/ml. Thirty larvae per dose were individually reared in a 30ml-plastic cup and checked daily the number of dead larvae. At 18 days post inoculation, the larvae were dissected and examined the cytoplasmic polyhedra in the mid-gut tissues under phase contrast microscope. The calculation of median lethal concentration(LC_{50}) and median lethal time(LT_{50}) value were followed by Finney's method(1972).

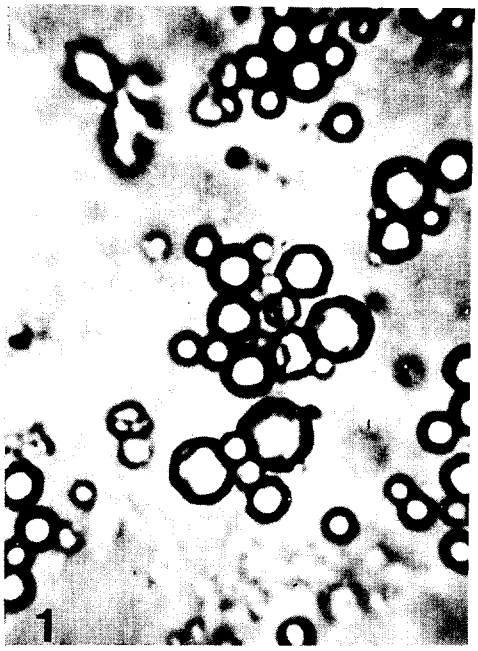
RESULTS AND DISCUSSION

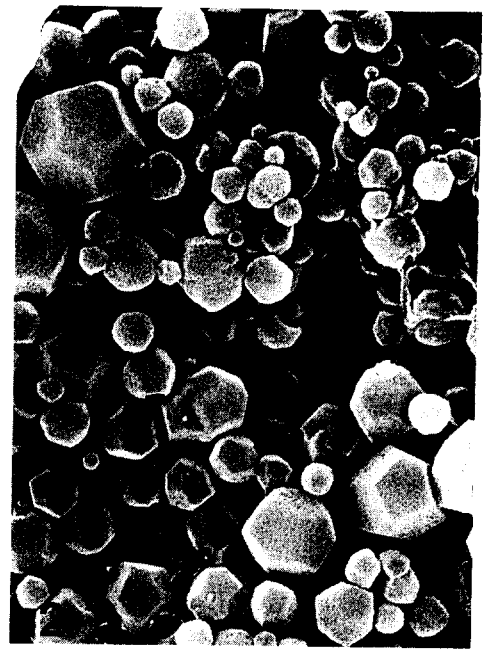
Morphology of HaCPV

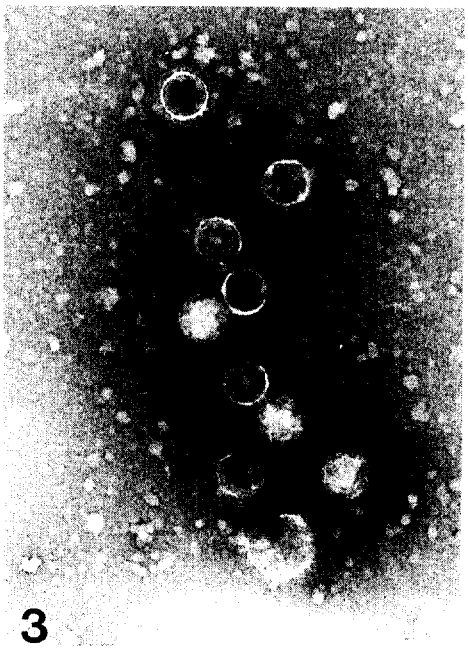
The shape of polyhedra was appeared hexagonal in outline under phase contrast microscope (Fig. 1) and definite icosahedral outline by the scanning electron microscope(Fig. 2). The polyhedra were variable in size with from 0.5 μm to 3.7 um. Hukuhara(1971) had reported five different shapes in *Bombyx mori* CPV according to the strains of the virus. Virus particles of the HaCPV were icosahedral symmetry with 55 nm in diameter and empty without nucleic acids in some particles(Fig. 3, 4). The size of virus particles of the HaCPV was similar to that of *Estigmene acrea* CPV(Langridge 1983), but were smaller than that of *Pectinophora gossypiella* CPV(Ignoffo & Adams 1966). Virus particles of *B. mori* CPV consist of the outer and inner icosahedral shell. The subunit of the outer shell consists of 5 smaller unit at its 5 corners and that of the inner shell is connected with the subunit of the outer shell (Hosaka & Aizawa 1964). As seen in Fig. 3, virus particles of the HaCPV were also consisted of 5 smaller subunit at corners like in *B. mori* CPV reported by Hosaka & Aizawa(1964).

Polyhedral protein

Polyhedral protein was compo[illegible]d of a major polypeptide with the mol. wt. of 24.3 Kd and 5 minors components in Fig. 5. Harrap & Payne (1979) had classified polyhedral proteins into different CPV groups. They determined the mol. wt. in the range of 25 Kd 30 Kd, i. e. 25 Kd in *Actias selenene* CPV and 28 Kd in *Spodoptera exempta* and *Manduca sexta* CPVs. Arella et al. (1984) reported the different mol. wt. of poly-







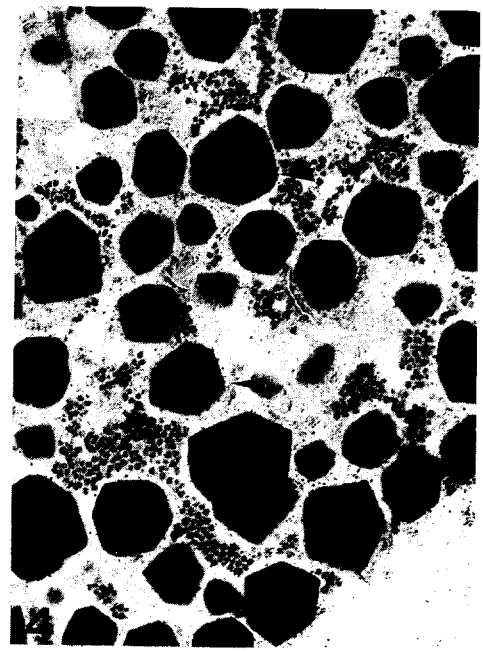


Fig. 1. Wet-mount preparation of *Heliothis assulta* cytoplasmic polyhedra as seen by phase contrast microscopic examination.

Fig. 2. Scanning electron micrograph of *Heliothis assulta* cytoplasmic polyhedra.

Fig. 3. Negative stain of virus particles of *Heliothis assulta* cytoplasmic polyhedrosis virus showing empty(E) and intact(I) contents.

Fig. 4. Matured polyhedra(arrow) in the sectioned midgut epithelial cell of *Heliothis assulta* larva infected with *H. assulta* cytoplasmic polyhedrosis virus.

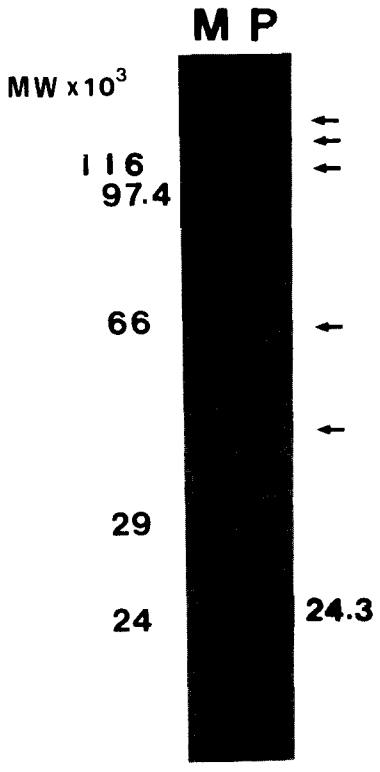


Fig. 5. SDS-PAGE of polyhedral protein purified from *Heliothis assulta* cytoplasmic polyhedrosis virus on 12.5% polyacrylamide gel.
(P) polyhedral protein, (M) molecular weight markers ; $\times 10^3$(116 : β-galactosidase, 97.4 : phosphorylase, 66 : albumin bovine, 29 : carbonic anhydrase, 24 : trypsinogen)

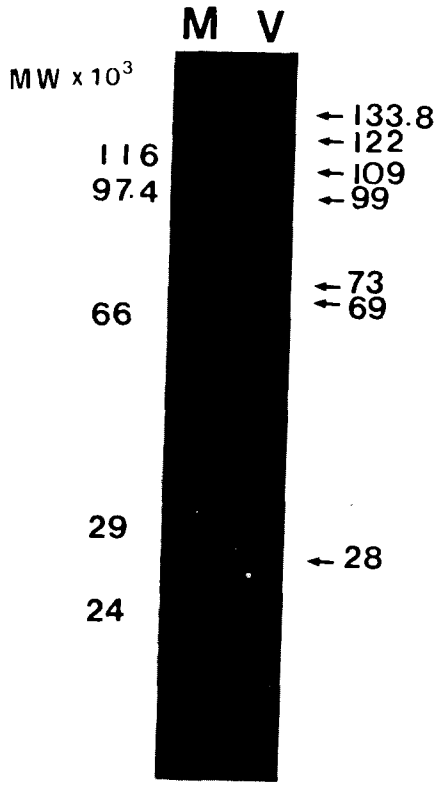


Fig. 6. SDS-PAGE of virus particle polypeptides of *Heliothis assulta* cytoplasmic polyhedrosis virus on 12.5% polyacrylamide gel. See the footnote in Fig. 5.

-hedral protein of *Euxoa scandens* CPV from larvae and from cell culture. He found the major polypeptide of 28 Kd from larvae and that of 31 Kd from cell culture. The mol. wt. of polyhedral protein from the HaCPV was determinded as smaller than other types of Lepidopteran species reported by Payne & Rivers(1976).

Polypetides of virus particles

Seven polypeptides of virus particles of the HaCPV ranging in the mol. wt. of 28.0-133.8 Kd were characterized by the SDS-PAGE(Fig. 6). The number of peptides of the particles was similar to that of *M. sexta* CPV (Galinskiet al. 1984) and the mol. wt. of virus particles in some CPV types I to V was larger than that in the HaCPV(Harrap & Payne 1979).

Viral RNA

The genomic RNA profiles observed in 3% polyacrylamide gels were presented in Fig. 7. The viral RNA was segmented with 10 double stranded RNA segments in the mol. wt. of 0.65Md-2.79Md and the total of mol. wt. was 18.08Md. Payne & Rivers(1976) grouped 29 of 33 isolates of CPVs into 11 virus types according to the similar RNA gel profiles. All eleven CPVs

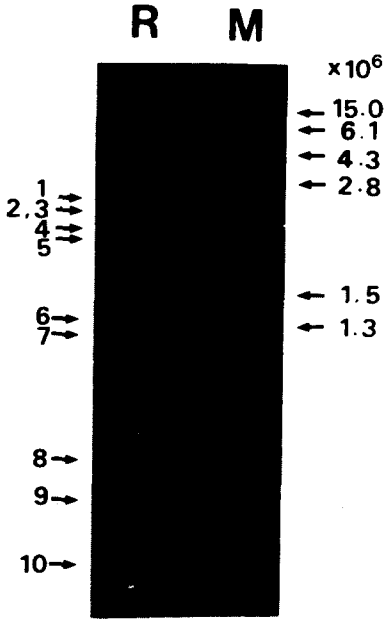


Fig. 7. SDS-PAGE of genome of *Heliothis assulta* cytoplasmic polyhedrosis virus on 3% polyacrylamide gel(R). Viral genome showing 10 segments (M). Molecular weight marker obtained from bacteriophage DNA digested with Hind Ⅲ.

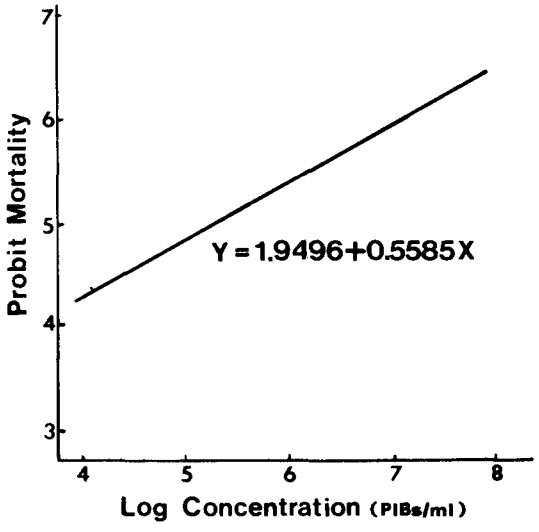


Fig. 8. The linear regression of *Heliothis assulta* cytoplasmic polyhedrosis virus on mortality probits in 3rd instar of *H. assulta* larvae.

Table 1. Median lethal concentration(LC_{50}) value to third instar *Heliothis assulta* larvae treated with *H. assulta* cytoplasmic polyhedrosis virus

Dose (PIBr/ml)[a]	Slope	Intercept	X^2	95% Fiducial limits Lower	Upper
LC_{50} 2.895×10^5	0.5585	1.9496	0.9495	8.810×10^3	9.484×10^6

[a] Ten larvae per dose(5.0×10^4 to 8 PIBs/ml) were individually tested with three replicates.

Table 2. Median lethal times(LT_{50}) value in third instar *Heliothis assulta* larvae treated with *H. assulta* cytoplasmic polyhedrosis virus

Dose (PIBs/ml)[a]	Slope	Intercept	X^2	LT_{50} (Days)
5.0×10^4	7.038	−4.301	0.119	20.96
$\times10^5$	14.843	−13.840	−0.004	18.59
$\times10^6$	11.830	−9.384	0.312	16.43
$\times10^7$	11.849	−8.947	0.490	15.03
$\times10^8$	7.646	−3.342	1.046	12.33

[a] See the footnote in Table 1.

into types contained 10 segments of RNA with a total mol. wt. of approx. 15×10^6(Lewandowski & Millward 1971, Payne & Tinsley 1974, Payne & Rivers 1976). In contrast, viral RNA of *Malacosoma disstria* CPV has 16 segments with an estimated mol. wt. of 20×10^6(Hayashi & Krywienzyk 1972). Particular notes for identification among types of CPVs are the differences from the mol. wt. of RNA and the number of polypeptides of virus particles.

Bioassay

Pathogenicity of the HaCPV which were examined for 18 days post inoculation (pi) against 3rd instar larvae of *H. assulta* was calculated at 2.895×10^5 PIBs/ml in the LC_{50} value (Table 1). In Fig. 8, the linear regression of lethal concentration was $Y=1.9469+0.5585\times(X^2\ 0.05=0.9465)$. The LT_{50} in the concentration of 5.0×10^7 PIBs/ml was 15.0days(Table 2). Infection period of the HaCPV was taken more days

than that of the nuclear polyhedrosis virus of *Spodoptera litura*(Im et al. 1988). Pathogenicity of *Lymantria dispar* CPV to 2nd instar larvae of *L. dispar* reported by Rollinson & Lewis(1973) was lower by 60-fold than that of the HaCPV to 3rd instar of *H. assulta* larvae. The sign of the virus disease was showed from 8 days pi and the mortality was the highest at 10 to 11 days pi. The symptoms of larvae infected with the HaCPV were loss of the appetite, the reduced body size the delay of larval periods, and the excretion of whitish fecal pellets, which are clumps of polyhedra, shortly before death or pupation. Magnoler(1974) found that the infection rate of *Porthetria*(*Lymantria*) *dispar* CPV was 96% to based on the presecnce of polyhedra in the tissues. And he also reported the pupal weights and adults emergence in infected insects with the CPV were significantly reduced.

Thus, the HaCPV would be a promisible agent for control of *H. assulta* larvae if production method of the HaCPV and utilization of the virus would be carried out in future.

REFERENCES CITED

Arella M., S. Belloncik & G. Devauchelle. 1984. Protein synthesis in a *Lymantria dispar* cell line infected by cytoplasmic polyhedrosis virus. J. Virol. 52(3) : 1024～1027.

Finney, D.J. 1971. Probit analysis. Cambridge Univ. Press, Cambridge.

Galinski, M.S., T. Kingan, Y. Rohmann, G.F., M.E. Martignoni & G.S. Beaudreau. 1984. Characterization of a cytoplasmic polyhedrosis virus infecting *Manduca sexta*. Intervirology 21 : 167～173.

Harrap, K.A. & C.C. Payne. 1979. The structural properties and identification of insect viruses. Adv. in Virus Res. 25 : 273～354.

Hayashi, Y. & J. Krywienzyk. 1972. Electrophoretic fraction of cytoplasmic-polyhedrosis virus genome. J. Invertebr. Pathol. 19 : 160～162.

Hosaka, Y. & K. Aizawa. 1964. The fine structure of the cytopiasmic-polyhedrosis polyhedrosis virus of the silkworm, *Bombyx mori* Linnaeus. J. Insect Pathol. 6 : 53～77.

Im, Dae Joon B.M. Shepard & R.M. Aguda. 1988. Pathogenicity and histopathology of a nuclear polyhedrosis virus of *Spodoptera litura*(Fab.). Insect Sci. Applic. 9(4) : 539～542.

Hukuhara, T. 1971. Variations in cytoplasmic-polyhedrosis virus. p.61～78. *In* H. Aruga & Y. Tanada (ed.), The cytoplasmic-polyhedrosis virus of the silkworm. Univ. of Tokyo Press, Tokyo.

Ignoffo, C. M. & J. R. Adams. 1966. A cytoplasmic -ploy hedrosis virus, *Smithiavirus pectinophorae* sp. n. of the pink ballworm, *Pectinophora gossypiella* (Saunders). J. Invertebr. Pathol. 8 : 59～66.

Katagiri, K. 1981. Pest control by cytoplasmic polyhedrosis viruses. pp 433～440. *In* H. D. Burges(ed.), "Microbial control of pests and plant diseases, 1970－1980." Academic Press, New York.

Laemmli, U.K. 1970. Cleavage of the head of bacteriophage T_4. Nature 227 : 680～685.

Langeridge, W.H.R. 1983. Characterization of a cytoplasmic polyhedrosis virus from *Estigmene acrea* (Lepidoptera). J. Invertebr. Pathol. 42 : 259～263.

Lewandowski, L.J. & S. Millward. 1971. Characterization of the genome of cytoplasmic polyhedrosis virus. J. Virol 7 : 434～437.

Magnoler, A. 1974. Effects of a cytoplasmic polyhedrosis virus on larval and postlarval stages of the gypsy moth, *Porthetria dispar*. J. Invertebr. Pathol. 23 : 263～274.

Maniatis, T.E., E.F. Fritsch & J. Sambrook. 1982. Molecular cloning. A laboratory manual. New York, Cold Spring Harbor Laboratory.

Martignoni, M. E. & P. J. Iwai. 1986. A catalog of viral diseases of insects, mites, and ticks. 4th ed. Gen. Tech. Rep. PNW-195. Portland. p. 51.

Matthews, R.E.F. 1982. Classification and nomenclature of viruses. Intervirology 17 : 1～199.

Neilson, M.M. 1964. A cytoplasmic polyhedrosis virus pathogenic for a number of Lepidopterous hosts. J. Insect Pathol. 6 : 41～52.

Payne, C.C. & C.F. Rivers. 1976. A provisional classification of cytoplasmic polyhedrosis virus based on the sizes of the RNA genome segments. J. Gen. Virol. 33 : 71～85.

Payne, C.C.M. Piasecka-Serafin & B. Pilley. 1977. The properties of two recent isolates of cytoplasmic polyhedrosis viruses. Intervirology 8 : 155～163.

Payne, C.C. & T.W. Tinsley. 1974. The structural

proteins and RNA components of a cytoplasmic polyhedrosis virus from *Nymphalis io*(Lepidoptera: Nymphalidae). J. Gen. Virol. 25 : 291～302.

Rollinson, W.D. & F.B. Lewis. 1973. Susceptibility of gypsy moth larvae to *Lymantria* spp. nuclear and cytoplasmic polyhedrosis viruses. Plant Protection. No. 124～125 : 163～168.

Van Regenmortal, M.H.V. 1990. Virus species, a much overlooked but essential concept in virus classification. Intervirology 31 : 241～254.

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