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A Stable Preservation of Extracellular Nonoccluded Virions from *Autographa californica* Nuclear Polyhedrosis Virus Infection

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Autographa californica 핵다면체 바이러스의 세포외 미봉입비리온의 안전한 보존

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A stable preservation method of extracellular non-occluded virion of *Autographa californica* nuclear polyhedrosis virus (AcNPV) was studied. AcNPVL-1 strain infected to *Spodoptera frugiperda* cell line and then the culture media were centrifuged. After centrifugation the supernatant containing extracellular nonoccluded virions of the AcNPV was harvested and incubated at 4°C. Even after the extracellular nonoccluded virions were incubated at 4°C for about 11 years, the infectivity and multiplication property of the nonoccluded virions in the *S. frugiperda* cell line were normal. However the titers of the nonoccluded virions in TC-100 medium measured about 11 years ago decreased from 8.9×10^7 to 3.8×10^5 pfu per m*l*. The AcNPV genome DNA fragment patterns from digestion with *Hind*III and *Eco*RI restriction endonucleases did not change. The AcNPV nonoccluded virions were stable at 4°C in the cultured medium of more than 10 years and the preservation of AcNPV nonoccluded virions at 4°C is easy and useful for handling.

Viruses are usually preserved at −70°C to −90°C in deep freezers or in liquid nitrogen (1). The techniques require very expensive instrument with auxilliary tools and buffers, also the stability and infectivity of the viruses are decreased very soon. When nuclear polyhedrosis viruses infected host cell lines, viral DNA replication occurs in the nuclei of invertebrate cells, and extracellular nonoccluded virions (NOV) bud out to the culture media (2-6, 10) and bundled nucleocapsids are occluded in polyhedral inclusion bodies (2-6). NOVs are responsible for the spread or infection from cell to cell via the invertebrate hemolymph *in vivo* of in cell culture media (2, 7-12). Therefore, it is necessary to study safe and easy preservation techniques of the extracellular NOVs.

Key words: Nuclear polyhedrosis virus, virus preservation
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Table 1. Titration of extracellular nonoccluded virions of *Autographa californica* nuclear polyhedrosis virus

Titration period	Titers of AcNPV NOVs by plaque assay[a]
11 years ago	8.9×10^7 pfu/m*l*[a]
11 years later	3.8×10^5 pfu/m*l*

[a]Lee and Miller (1978).

Autographa californica nuclear polyhedrosis virus L-1 strain was multiplied in *Spodoptera frugiperda* cell line (13) and then the infected cells were pelleted. The extracellular NOVs of *A. californica* nuclear polyhedrosis virus in the supernatant cultured TC-100 medium (11) were incubated at 4°C for about 11 years.

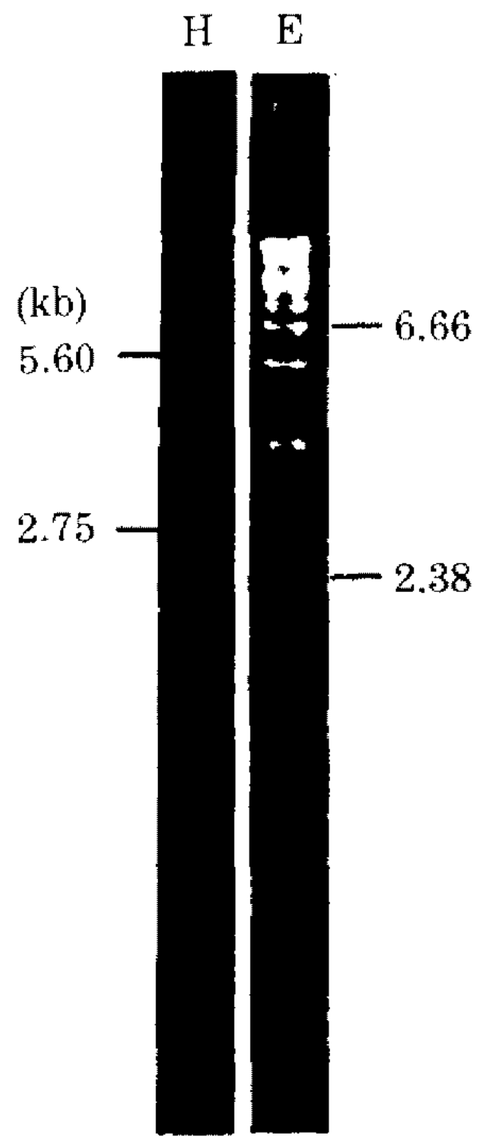

Fig. 1. *Hind*III (lane H) and *Eco*RI (lane E) restriction fragment patterns of *Autographa californica* nuclear polyhedrosis virus genome DNA.

Recently the infection and titration of the NOVs of the *A. californica* nuclear polyhedrosis virus was carried out by the procedure of Lee and Miller (10). The infectivity and multiplication of the NOVs in the *S. frugiperda* cell line were normal, however the titers of the NOVs decreased from the 8.9×10^7 to 3.8×10^5 pfu per m*l* (Table 1). Also the restriction patterns of the genome DNA was prepared by the Lee and Miller method (10); however, they were the same to the former results of Lee and Miller (10). The results indicate that the nonoccluded virions of AcNPV are stable at 4°C in the cultured medium for over 10 years and the preservation of AcNPV NOVs at 4°C is easy and useful for handling.

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요 약

Autographa californica nuclear polyhedrosis virus (AcNPV) L-1주의 extracellular nonoccluded virion (NOV)을 보관하는 방법을 연구하였다. AcNPV NOVs을 *Spodoptera frugiperda* cell line에 감염을 시킨 후에 배양액을 원심분리하여 AcNPV NOVs가 들어 있는 상등액을 취하여 4℃에서 약 11년간을 보관하였다. 보관되어 있는 AcNPV NOV을 *Spodoptera frugiperda* cell line에 재감염하여 관찰한 결과 NOVs의 감염과 증식이 정상적이었으며, NOV의 역가가 8.9×10^7 pfu/m*l*에서 3.8×10^5 pfu/m*l*로 떨어졌을 뿐이다. 또한 *Hin*dIII와 *Eco*RI 제한효소로 AcNPV genome DNA을 절단하여 패턴을 조사한 결과 DNA 제한효소 패턴은 변하지 않았다. 즉 AcNPV NOVs는 4℃에서 보존하면 10년 이상 안정성이 있고, 취급이 용이하다는 것을 알 수 있었다.

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