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Molecular cloning and foreign gene expression of restriction endonuclease fragments of the Hc nuclear polyhedrosis virus DNA

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Hc nuclear polyhedrosis virus DNA genome was digested with *Eco*RI endonuclease, these partial fragments were recombined into the pUC8 plasmid vector and transformed in *E. coli* JM 83 cell. The genome DNA has 24 *Eco*RI fragments and 12 fragments of them were subcloned. The four recombinants were named as eNP-O, eNP-Q, eNP-R and eNP-S. The expression of foregin gene of the recombinants was investigated by analysing protein patterns on the SDS-PAGE. The eNP-O, eNP-Q and eNP-R were expressed a different weight of protein as comparision with polypeptide bands of *E. coli* JM 83 host cell.

Key Words : Baculovirus, Transformation, Recombinants, pUC8 vector, *E. coli* JM 83 cell, Gene expression

The nuclear polyhedrosis viruses (NPVs) belong to a subgrouped genus Baculovirus, which viral genomes have a relatively large molecular weight of covalently double stranded DNA ranging in size from 70 to 100×106 daltons (Bugess, 1977; Cochran *et al.*, 1982; Summers and Anderson, 1973). These viruses have been found to infect cultured penaeid shrimps, *Penaeus monodon* and *P. japonicus* and to cause occasionally high mortality of the populations (Lightner, 1983; Tsing and Bonami, 1984; Lightner and Redman, 1981). The physical map of NPVs genome DNA was made by using restriction enzymes (Cochran *et al.*, 1982; Smith and Summers, 1978). Cochran *et al.* (1982) subcloned almost the entire genome of Autographa californica (Ac) NPV as *Bam*HI, *Eco*RI and *Hind*III fragments in the plasmid pBR322. For more detailed analysis of the genome of the NPV and its relationship with other members of the NPV, nucleotide sequence was analyzed to acquire the basic data on the development of HcNPV expression vector system. For these purposes it was necessary to clone the HcNPV DNA.

In this study, I describe the partial cloning of the *Eco*RI restriction DNA fragments of HcNPV DNA genome in *E. coli* plasmid vector, pUC8. And the four transformants of them were analyzed.

Materials and methods

Virus culture and transformation : The HcNPV inoculum (Lee and Lee, 1988) used was virions derived from a cell culture medium. *Spodoftera frugiperda* continuous cell line (IPLB-SF-21) (Vaughn *et al.*, 1977) was used for propagation of the virus. The cells were routinely maintained in TNM-FH medium. *E. coli* JM 83 host cell was used in transformation. And isolation of transformant was carried out by method of Summers and Smith (1987). Lamda bacteriophage DNA digested with *Bst*EII was used as a size marker.

Preparation of viral DNA and construction of recombinant plasmids : Isolation and purification procedures of HcNPV and HcNPV DNA were carried out with the methods of Cochran *et al.* (1982). And the DNA was digested with *Eco*RI restriction endonuclease as recommended by the manufacture's instruction (Bethesda Research Lab.). The construction of the recombinant plasmids and the transformation in *E. coli* JM 83 cell were carried out by the method of Maniatis *et al.* (1982).

Purification of plasmid DNA : Both *E. coli* JM 83 containing the plasmid pUC8 and recombinant plasmids were grown in either 5 to 10 ml (miniprep) or 50 ml of Luria broth containing 20 μg of the ampicillin per ml. Cell suspensions were grown at 37℃ in a shaker bath and the cells were harvested and a cleared lysate was prepared as described by Guerry *et al.* (1973) with some modifications. After addition of fresh lysozyme (Sigma Co.) to 1.5 mg/ml, the suspension was brought to 1% (wt/vol) SDS and 1 M NaCl. Lysates from 50 ml cultures were incubated on ice overnight and centrifuged at 17,000 × g for 45 min at 4℃. Minipreps were left on ice for 45 min and centrifuged (15,000 × g) at 4℃. The supernatant was designated the cleared lysate. To purify candidate recombinant plasmids from minipreps 0.5 ml volumes of cleared lysates were extracted sequentially with phenol and chloroform in eppendorf tubes. The DNA was then precipitated with 2.5 volumes ethanol at -70℃ for 30 min, and the precipitate (5 to 10μg of plasmid DNA) was dissolved in 50 $\mu\ell$ of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5).

SDS - polyacrylamide gel electrophoresis : Electrophoresis was carried out using a 10% polyacryamide vertical slab gel in the presence of 0.1% SDS as described by Laemmli (1970). After electrophoresis, the gel was stained with Coomassie brilliant blue R-250. Molecular weight standard proteins (Pharmacia Co.) were used in each playing.

Results and Dicussion

DNA analysis : The DNA fragments were fractionated in agarose gel as shown in Fig. 1. The HcNPV genome DNA has 24 *Eco*RI cleavage sites. The size of the HcNPV genome DNA was approximately 127.6 Kilobases. Therefore, the results showed similar to fragment patterns to AcNPV E-2 by the report of Cochran *et al.* (1982). The AcNPV E-2 genome DNA had a 24 *Eco*RI cleavage sites and molecular weight was about 128.0 Kb. These numbers of HcNPV fragments are similar to

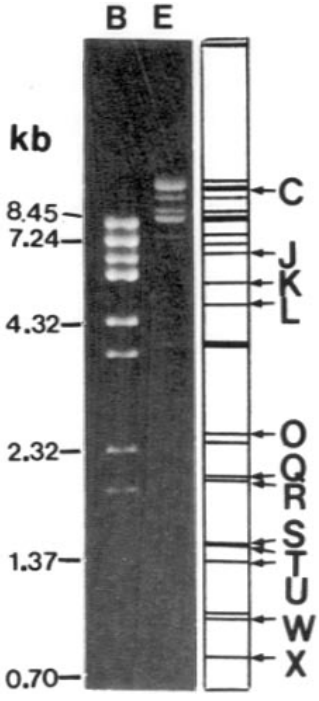

Fig. 1. Agarose gel electrophoresis patterns of NPV DNA after digestion with *Eco*RI restriction endonuclease (E). Lamda DNA digested with *Bst*E II was used as size marker (B). The arrows indicate cloned fragments.

those of AcNPV genome DNA (Smith and Summers., 1978), but the sizes of fragments are slightly different. The individual DNA fragments were assignated an alphabetical designation on the basis of molecular sizes such that the largest fragment for enzyme was referred to as A and the second largest was referred to as B, etc.

Construction and expression of recombinants : After transformation to the *E. coli* strain JM 83, bacterial clones were screened for the ampicillin and X-gal phenotype. The HcNPV genome DNA has 24 *Eco*RI fragments and 12 fragments of them were subcloned (Fig. 1). Fig. 2 shows examples of recombinant plasmids containing HcNPV DNA *Eco*RI fragments. The recombinants were named by an eNP- alphabetical ordering letter on the basis of the molecular sizes cloned in the vector i.e. the

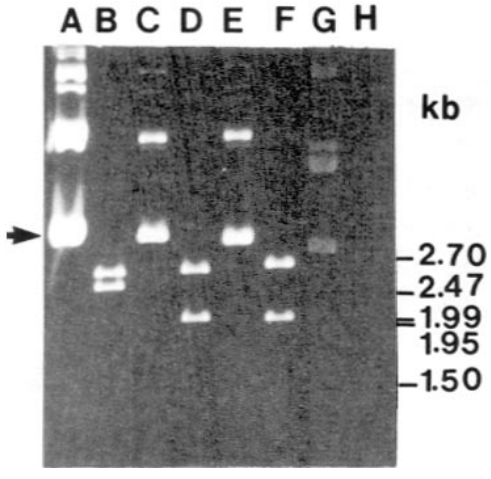

Fig. 2. Agarose gel electrophoresis patterns of DNAs from recombinants and their redigested patterns. Lane A is eNP-O DNA, Lane B is eNP-O DNA/*Eco*RI, Lane C is eNP-Q DNA, Lane D is eNP-Q DNA/*Eco*RI, Lane E is eNP-R DNA, Lane F is eNP-R DNA/*Eco*RI, Lane G is eNP-S DNA, Lane H is eNP-S DNA/*Eco*RI, The arrow indicates ccc DNA.

F fragment is named as eNP-F, etc. There are four clones i.e. eNP-O (lane A), eNP-Q (lane C), eNP-R (lane E) and eNP-S (lane G). The expression of foreign gene of four recombinants was investigated by analyzing protein patterns on SDS-PAGE (Fig. 3). Recombinants, eNP-O,

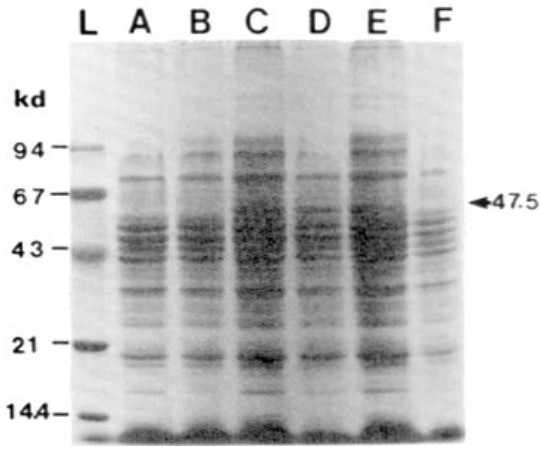

Fig. 3. SDS-polyacrylamide gel electrophoresis patterns of total protein of recombinants. L : Low molecular weight standards (kd), A : *E. coli* JM 83 cell, B : *E. coli* JM 83 (pUC8) cell, C : eNP-O, D : eNP-Q, E : eNP-R, F : eNP-S.

eNP-Q and eNP-R were appeared one of unknown protein peptide band as comparison with polypeptide bands of *E. coli* JM 83 host cell. The molecular weight of protein band was about 47.5 Kd. This result is needed to study continously. Cochran *et al.* (1982) reported that library containing AcNPV DNA restriction fragment was constructed by using the pBR322 plasmid as a vector, using restriction enzymes with *Eco*RI, *Hind*III, *Pst*I, *Xho*I, *Bam*HI, *Sma*I. The gene library was showed more than 95% of the viral genome, consist of 2 of the 7 *Bam*HI fragments, 12 of the 24 *Hind*III fragments and 23 of the 24 *Eco*RI fragments.

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Hc nuclear polyhedrosis virus DNA 제한효소절편의 molecular cloning 과 외래 유전자 발현

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HcNPV DNA genome 을 제한효소 *Eco*RI 으로 절단하여 그들의 일부 절편을 pUC8 vector 에 cloning 한 후 *E. coli* JM 83 세포에 형질 전환시켰다. 이 결과 24 개의 *Eco*RI 절편중 12 개의 절편이 cloning 되었다. 이들 제조합체중 4 개는 eNP-O, eNP-Q, eNP-R, eNP-S 라 명명하였다. 또한 이들 제조합체의 외래 유전자 발현을 SDS-PAGE 에 의해 단백질 패턴을 분석하였다. 그 결과 제조합체 eNP-O, eNP-Q, eNP-R 에서는 *E. coli* JM 83 숙주세포의 단백질 밴드와 비교하여 다른 분자량을 갖는 밴드가 나타났다.

Key Words : Baculovirus, Transformation, Recombinants, pUC8 vector, *E. coli* JM 83 cell, Gene expression