RESULTS AND DISCUSSION

1. Restriction pattern analysis of Thai BmNPV

1.1 Extraction of whole genomic BmNPV DNA

Determining the restriction enzyme pattern of the viral DNA is an essential tool in identifying and distinguishing isolates of virus. High quality and quantity of DNA are needed to demonstrate the restriction profile of each virus isolate. The BmNPV DNA extraction method used in this study following the method of Chaeychomsri (2003), provided a prominent DNA band on the gel (Figure 8). It is a single clear band with no interference of RNA. The obtained BmNPV DNA was in high quantity and quality and can be used to demonstrate the differences of the restriction patterns when digested with different enzymes.

There were many methods to harvest the virus, BmNPV from the insect body. BmNPV was collected from the insect haemolymph by cutting prolegs before filtering through layer of gauze (Singh *et al*., 1996). BmNPV was also collected by plaque purification (Hashimoto *et al*., 1994; Maeda and Majima, 1990). In this study, the virus was collected from infected larvae by allowing the insect body to putrefy and release polyhedra naturally. This method of viral harvesting was rather convenient to practice comparing with other methods. Viral DNA extraction described in this study was quite similar to previous methods (Maeda and Majima, 1990; Hashimoto *et al*., 1994; Singh *et al*., 1996) in which the main chemical used were alkaline solution for solubilizing the occlusion bodies and followed by proteinase K and SDS for releasing viral DNA. BmNPV DNA extraction used by various researchers were different in a few steps. For example, there was step of dialysation the DNA with SSC after extraction with phenol-chloroform-isoamyl alcohol in the study of Singh *et al*. (1996) while this step was omitted by others.

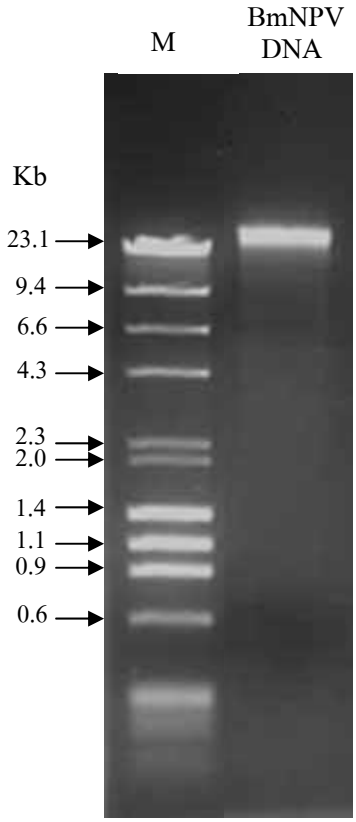

Figure 8 Gel electrophoresis of the DNA of Thai BmNPV extracted by the method of Chaeychomsri (2003).
M= marker : λ DNA- *Hin*dIII and ϕ X174 DNA-*Hae*III Mix

1.2 Restriction pattern of Thai BmNPV

The restriction pattern of BmNPV DNA was demonstrated in Figure 9. When the BmNPV DNA was cut with *Bam*HI, *Bgl*II, *Hin*dIII, *Nco*I and *Pst*I, 6, 11, 19, 11 and 17 DNA bands were observed on the gel, respectively. Molecular size of each DNA fragment was analyzed by comparison with the migration of the size marker and the fragments were assigned an alphabetical designation based on size as shown in Table 1. The biggest bands were 26.0 kb of fragment A and B digested with *Bam*HI. The smallest band that could observe was 0.3 kp of

fragment S digested with *Hin*dIII. The Thai BmNPV genome digested with *Bam*HI, *Bgl*II, *Hin*dIII, *Nco*I and *Pst*I was measured approximately 92.3, 102.6, 117.9, 114.3 and 125.8 kb, respectively. It was estimated to be in the range of 92.3-125.8 kb with the average of 109.8 kb. Genome estimation was based on the restriction fragments appeared on the gel. Some fragments may contain DNAs with little different in molecular size, therefore, they co-migrated in the same distance resulting in the overlapping DNA bands that cannot be differentiated. Therefore, bands which were more intense than the regular single bands were considered as two fragments of approximately same size.

Restriction patterns of genome of different strains of BmNPV had been previously reported. Maeda and Majima (1990) revealed the restriction pattern and genome size of BmNPV T3 isolate from Japan. The DNA was digested with *Eco*RI, *Hin*dIII, *Pst*I, *Bam*HI, *Kpn*I and *Sma*I. By summing the size of the fragments generated by these enzymes, the entire genome was estimated to be 130 kb. Hashimoto *et al.* (1994) showed restriction pattern of BmNPV D1 isolate from Japan digested with *Apa*I, *Bam*HI, *Bgl*I, *Hin*dIII, *Kpn*I and *Xho*I and the genome size was estimated to be 126.4 kb. Singh *et al.* (1996) reported the genome size of BmNPV N isolate from India as estimated from the restriction fragments of the genome digested with *Hin*dIII and *Eco*RI was measured approximately 118 kb. For complete genome of BmNPV, only T3 isolate was submitted in GenBank accession number L33180 and the genome was 128,413 nucleotides long (Gomi *et al*., 1999).

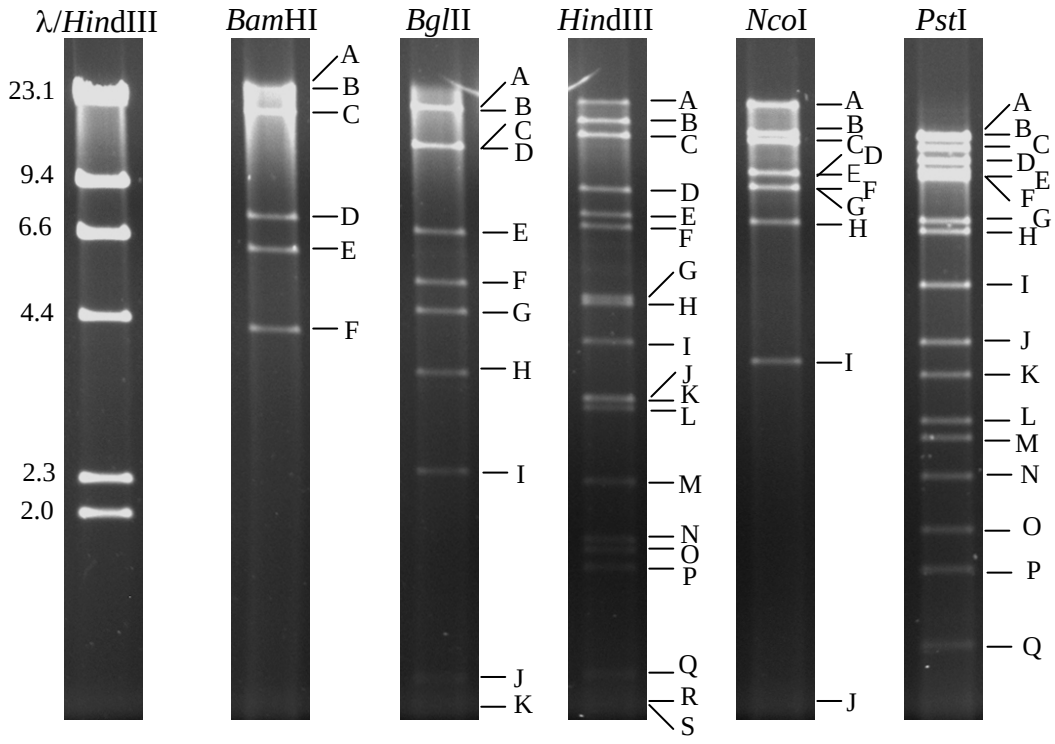

Figure 9 Cleavage patterns of Thai BmNPV DNA using the restriction endonuclease *Bam*HI, *Bgl*II, *Hin*dIII, *Nco*I and *Pst*I. Viral DNA was digested with these endonucleases and the cleaved fragments were separated on 0.6% SeaKem GTG agarose gel. Lane marked λ/*Hin*dIII showed molecular size marker pattern in kb. Each visible fragment was assigned a letter as shown.

Table 1 Restriction endonuclease cleavage fragments of the Thai *Bombyx mori* nucleopolyhedrovirus DNA

Fragment	Size (kb) of BmNPV restriction fragments				
	*Bam*HI	*Bgl*II	*Hin*dIII	*Nco*I	*Pst*I
A	26.0	22.9	23.1	22.3	17.4
B	26.0	22.9	20.1	17.9	17.4
C	22.5	16.2	17.8	16.5	15.4
D	7.5	16.2	9.2	11.6	13.1
E	6.1	7.1	7.9	11.6	11.2
F	4.2	5.5	7.3	9.3	10.4
G		4.7	4.9	9.3	7.4
H		3.7	4.8	7.5	6.8
I		2.4	4.1	7.5	5.3
J		0.6	3.4	3.8	4.1
K		0.4	3.4	0.4	3.7
L			3.2		3.1
M			2.3		2.9
N			1.8		2.4
O			1.7		2.0
P			1.5		1.8
Q			0.7		1.4
R			0.4		
S			0.3		
Total	92.3	102.6	117.9	114.3	125.8

Table 2 illustrated restriction endonuclease cleavaged fragments of Thai BmNPV DNA and DNA of other isolates of BmNPV such as T3 and D1 from Japan, and N from India. T3 and T3* are the same isolate but the fragment sizes of T3 were from the report of Maeda and Majima (1990) while fragment sizes of T3* of L33180 complete genome sequence were analyzed by computer software.

*Bam*HI restriction patterns showed that there were at least 6 DNA bands for BmNPV of Thai and T3 isolate while there were at least 5 DNA bands for the D1 isolate. Three small fragments digested with *Bam*HI of all BmNPVs were in similar size. The smallest fragments of Thai, T3, T3* and D1 were 4.2, 3.9, 4.2 and 4.2 kb, the next smaller fragments were 6.1, 6.0, 6.2 and 6.0 kb and the small fragments were 7.5, 7.3, 7.6 and 7.5 kb. When comparing fragment size of the Thai and T3 isolates, there were four fragments which gave the similar size, C, D, E and F. The largest fragment of Thai isolate was smaller than T3 and D1 isolate. The size marker used in this study was the low molecular weight size marker which perhaps too low and in narrow range until could not illustrate the DNA fragments that were bigger than 23.1 kb.

*Hin*dIII restriction patterns showed there were at least 19 bands for BmNPV of Thai and N isolate while there were at least 18 and 20 bands for the T3 and D1 isolate, respectively. DNA fragments of the Thai isolate were different in size from those of the T3, D1 and N isolate. In comparison between DNA fragments of the Thai and T3 isolate digested with *Hin*d III, fragment E, G, H, M, N, O, P and R of the Thai isolate were similar to fragment F, I, J, N, O, P and R of T3 isolate, respectively. Some DNA fragments of the Thai isolate were similar to fragments of D1 isolate. For example, fragment D, L, M, N and Q of the Thai isolate were similar to fragment D, M (N), O, P and T of the D1 isolate. In addition, there were some fragments of Thai isolate which were similar to fragment of N isolate. For example, fragment G, H, I, L, M, N and O of the Thai isolate were similar to fragment H, I, J, K, M, P and Q of N isolate.

*Pst*I restriction patterns showed there were at least 17 bands for BmNPV of Thai isolate while there were at least 19 bands for the T3 isolate. When digested BmNPV DNA with

*Pst*I, fragment A(B), G, M, N and O of Thai isolate were similar to fragment A(B), F(G), M, N(O) and P of the T3 isolate.

Even though T3 and T3* of BmNPV are the same isolate, some digested DNA fragments were different. For example, fragment A of T3 isolate digested with *Bam*HI was 54 while that of the T3* isolate was 51.1 kp and fragment A of T3 isolate digested with *Hin*dIII was 30 while that of T3* isolate was 27.3 kb. In addition, Fragment D of T3 isolate digested with *Pst*I was 12.5 kp while that of T3* isolate was 11.8 kb. This comparison study indicated that there were molecular size differences of the DNA fragments digested with restriction enzymes among or within viral isolates. The differences may result from the method of analysis.

In this study, size of the DNA fragments was estimated by comparison with the fragments of DNA marker that electrophoresed on the gel. This method was also used to estimate restriction fragments of the N isolate, while DNA fragments obtained from T3 and D1 isolate were estimated from the clone fragments which were probed by hybridization. Therefore, genome of Thai and N isolate were similar in size when digested with *Hin*dIII while the Thai BmNPV genome was smaller than that of the T3 and D1 isolate. Hence, DNA fragments with similar size of the T3, and D1 isolate can be distinguished while DNA fragments of the Thai isolate that have similar size cannot be clearly separated by gel electrophoresis. Even though bands which more intense than the regular single bands were considered as two fragments of same size, sometimes there were more than two of similar size fragments of fragment so it was difficult to determine correctly. However, this is only the preliminary investigation in order to compare restriction pattern of BmNPV with other isolates of previous reports. For intensive study of the whole genome, the co-migrated fragments should be elucidated by hybridization with different probes on the overlapping bands. However, the process comprises of several complicate work, is time consuming and expensive

The results showed that restriction enzyme that gave more fragments can be used for differentiation of isolate better than restriction enzyme that gave a few fragments. For example, *Hin*dIII can give more polymorphism of BmNPV isolates than *Bam*HI.

There were many reports on restriction pattern of other NPVs. Smith and Summers (1978) reported that restriction pattern of DNA of several NPVs (*Autographa californica* NPV, *Orgya pseudotsugata* NPV, *Rachiplusia au* NPV, *Portheria dispar* NPV, *Spodoptera exiqua* NPV, *Porthetria dispar* NPV, *Trichoplusia ni* NPV, *Heliothis zea* NPV, *H. armigera* NPV) can identify isolates of baculoviruses. Moreover, Lavina-Caoili *et al*. (2001) studied restriction pattern of 10 isolates of *Spodoptera litura* NPV from Japan, China and Philippines. The 10 isolates of SlNPV displayed similar overall restriction endonuclease pattern except for deletion or insertion of a few DNA fragments, indicated that there were minor differences among isolated genotypic variants in their genome organization. Restriction endonuclease analysis of a number of baculoviruses from different geographical regions has shown that each isolate has a unique set of DNA fragments which may indicate the variation in the genomic DNA sequence. Therefore, analysis of viral DNA by digesting with different restriction endonucleases and observing the restriction profiles is convenient, economy and one of useful tools in identification and classification of the viruses.

Table 2 Restriction endonuclease cleavaged fragments of the Thai BmNPV DNA and DNA of the other isolates of BmNPV*

Fragment	Size (kb) of BmNPV restiction fragment											
	*Bam*HI				*Hin*dIII					*Pst*I		
	Thai	T3	T3*	D1	Thai	T3	T3*	D1	N	Thai	T3	T3*
A	26.0	54	51.4	58.1	23.1	30	27.7	29.5	24.06	17.4	17.5	17.8
B	26.0	36	37.3	50.5	20.1	17	16.9	17.0	15.16	17.4	17.5	17.5
C	22.5	22	21.8	7.5	17.8	15.5	15.5	10.2	14.80	15.4	17.0	16.6
D	7.5	7.3	7.6	6.0	9.2	10.0	9.7	9.0	9.44	13.1	12.5	11.8
E	6.1	6.0	6.2	4.2	7.9	8.9	8.9	8.1	8.56	11.2	10.8	10.2
F	4.2	3.9	4.2		7.3	7.8	8.2	8.1	8.20	10.4	7.2	7.3
G					4.9	7.8	8.1	6.5	5.16	7.4	7.2	7.2
H					4.8	5.8	5.9	5.5	4.90	6.8	5.5	5.5
I					4.1	5.1	5.0	5.0	4.70	5.3	5.4	5.4
J					3.4	4.8	4.9	4.9	4.00	4.1	5.4	5.3
K					3.4	3.8	3.9	4.2	3.18	3.7	4.9	4.9
L					3.2	3.1	3.2	3.5	3.11	3.1	4.6	4.7
M					2.3	3.0	3.0	3.2	2.29	2.9	2.8	2.7
N					1.8	2.2	2.3	3.2	2.19	2.4	2.3	2.4
O					1.7	1.7	1.8	2.3	1.90	2.0	2.3	2.4
P					1.5	1.5	1.6	1.8	1.83	1.8	1.9	1.9
Q					0.7	1.0	1.0	1.7	1.74	1.4	1.5	1.6
R					0.4	0.7	0.8	1.0	1.23		1.5	1.6
S					0.3			1.0	1.05		1.3	1.3
T								0.7				
Total	92.3	129.2	128.5	126.3	117.9	129.7	128.4	126.4	117.5	125.8	129.1	128.1

* T3 = BmNPV isolate from Japan (Maeda and Majima, 1990)

T3*= BmNPV isolate from Japan (Gomi *et al*., 1999)

D1 = BmNPV isolate from Japan (Hashimoto *et al*., 1994)

N = BmNPV islate from India (Singh *et al*., 1996)