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# Characterization of the genome of *Oryctes* baculovirus, a viral biocide of the insect pest *Oryctes rhinoceros*

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**Abstract.** *Oryctes* baculovirus is a viral biocide exploited for the control of the insect pest *Oryctes rhinoceros.* We have recently established a physical map of the genome of the Indian isolate of *Oryctes* baculovirus (OBV-KI). Here we examine the genomic relatedness between OBV-KI and OBV-PV505, the type isolate (originally from the Philippines), by DNA reassociation kinetics and by the use of restriction endonucleases. On the basis of differences in restriction-enzyme profiles between the two genomes, and previously reported differences in protein profiles and antigenic makeup, we propose the taxonomic status of a variant of *Oryctes* baculovirus for the Indian isolate.

**Keywords.** Baculovirus genome; *Oryctes baculovirus; Oryctes rhinoceros*; viral biocide.

## 1. Introduction

*Oryctes* baculovirus (OBV) infects the rhinoceros beetle *Oryctes rhinoceros* L., a major insect pest of coconut and oil palm found throughout the tropics. OBV has been successfully used as a viral biocide against *O. rhinoceros* in the islands of the South Pacific and the Indian Ocean (Bedford 1981) and against *O. monoceros* in East Africa (Purrini 1989). OBV was isolated for the first time from a wild population of *O. rhinoceros* in Malaysia (Huger 1966) and subsequently from Indonesia and the Philippines (Zelazny 1977). Another isolate of OBV, OBV-KI, was obtained from a wild population of rhinoceros beetles in India, in Kerala state (Mohan *et al* 1983). This virus isolate has been used successfully for biological control of rhinoceros beetles and their larvae in the Lakshadweep Islands (Mohan 1990). OBV is a nonoccluded baculovirus, and is distinguished from the other subgroups by the absence of polyhedrin or granulin, the matrix proteins (Matthews 1982).

Characterization of the genome and the constituent proteins of the OBV type isolate from the Philippines (PV505) has been reported (Payne 1974; Crawford and Sheehan 1985; Crawford *et al* 1985). Recently we constructed a physical map of OBV-KI DNA (Mohan and Gopinathan 1991). We have also shown that there are significant differences in the protein profile and antigenic cross-reactivity between KI and PV505 (Mohan and Gopinathan 1989a, b). Here we report a detailed characterization of the OBV-KI genome and its comparison with that of the type isolate, OBV-PV505.

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Abbreviations used: OBV, *Oryctes* baculovirus; OBV-KI or KI, Kerala isolate of OBV; OBV-PV505 or PV5O5, Philippine isolate of OBV; OBV-MI, Malaysian isolate.

## 2. Materials and methods

### 2.1 *Viruses*

OBV-KI was isolated from a wild population of *O. rhinoceros* in Kerala state, India (Mohan *et al* 1983). Isolate PV505, originally from the Philippines, was obtained from Allan Crawford, DSIR, Auckland, New Zealand. Both isolates of OBV were routinely propagated and multiplied in *O. rhinoceros* beetles. Viral particles voided in the excreta of infected beetles were purified by a series of ultracentrifugations in buffered sucrose gradients (Mohan and Gopinathan 1989a). The morphology of OBV-KI particles was studied by electron microscopy of midgut epithelial tissue of beetles infected in the laboratory.

### 2.2 *Restriction analysis of DNA, homology*

Virus purified by sucrose gradient centrifugation was disrupted by overnight incubation in a solution of 0·1 M Tris–HCl (pH 8·0), l0mM EDTA, 0·1% SDS and l00$\mu$g/ml proteinase K. Disrupted virus was extracted three times with phenol: chloroform : isoamyl alcohol (25:24:1), and the DNA was precipitated with ethanol by Standard methods (Maniatis *et al* 1982). The DNA was hydrolysed in 90% formic acid. Base composition was analysed by HPLC using a Lichrosorb RPC-18 column (Patel and Gopinathan 1987).

Purified DNA from KI or PV505 (2 $\mu$g) was restricted with various endonucleases and the DNA fragments were routinely resolved by electrophoresis in 0·8% agarose gels. For better estimation of restriction-fragment sizes, percentage of agarose or duration of electrophoresis was varied to resolve different size ranges. DNA fragment sizes were computed using the Sequaid program (Pharmacia), with $\lambda$ DNA HindIII restriction fragments as markers.

Sequence homology between KI and PV505 DNAs was determined by allowing denatured labelled KI DNA to reanneal with an excess of denatured unlabelled PV505 DNA. Self-reassociation of KI DNA and reassociation between labelled KI DNA and the nonhomologous salmon sperm DNA were also determined as controls. A sample of sheared KI DNA was labelled with $^{32}$P by nick translation (Rigby *et al* 1977). The kinetics of reassociation between labelled and unlabelled KI DNA strands and between labelled KI DNA and unlabelled PV505 DNA or salmon sperm DNA were analysed under the conditions described by Wagner and Paschke (1977), except that S1 nuclease was used to digest the unreannealed fraction (Rohrmann *et al* 1978).

## 3. Results and discussion

Ultrathin sections of KI-infected midgut epithelial cells of *O. rhinoceros* contained bacilliform, enveloped virus particles measuring 220–240 nm × 80–110 nm (figure 1). Nucleocapsids of OBV-KI are surrounded by a distinct envelope (arrows) composed of trilaminar membrane. Since the nucleocapsid is loosely enclosed by the envelope, the enveloped virions assumed various shapes and sizes. For this reason, negatively stained preparations were found to be unsuitable for virus size

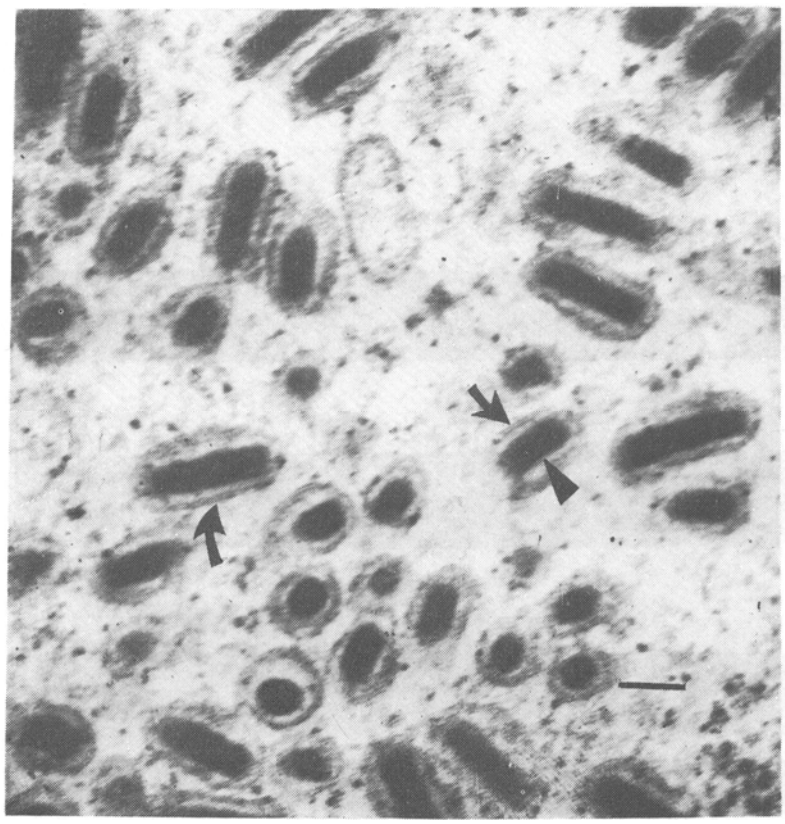

**Figure 1.** Morphology of OBV-KI. The arrowhead points to a nucleocapsid and the arrows show the membrane envelope. Slices of virus-infected midgut were fixed in 2% glutaraldehyde stained with 1% $OsO_4$, and finally embedded in 'Epon-Araldite'. Ultrathin sections were examined in a transmission electron microscope. Bar represents 100 nm.

determination. However, the morphology and dimensions of OBV-KI in the electron micrographs of infected-tissue sections agree well with the published data on OBV-PV505 (220 nm × 120 nm; Payne 1974) and OBV-MI (195 nm × 70 nm; Huger 1966). Morphologically OBV-KI is indistinguishable from the other OBV isolates.

The DNA isolated from OBV-KI gave a single sharp band of high $M_r$ when subjected to agarose gel electrophoresis. The base compositions of KI and PV505 DNAs determined from thermal melting analysis or by direct base-composition analysis corresponded to 43·41 mol% G + C.

The approaches used for studying genetic relatedness among baculoviruses include DNA hybridization, restriction analysis, and comparison of polyhedrin amino acid and nucleotide sequences (Mclntosh *et al* 1987). Reassociation kinetics showed hardly any difference between the homologous reassociation of KI DNA strands (renaturation expression, $C_0t_{½}$ 0·168 mol sec $lit^{-1}$) and the heterologous reassociation of KI and PV505 strands ($C_0t_{½}$ 0·175 mol sec $lit^{-1}$), indicating a high degree of sequence homology between the DNAs of the two isolates. The two DNAs could also not be differentiated on the basis of melting temperature ($T_m$) and

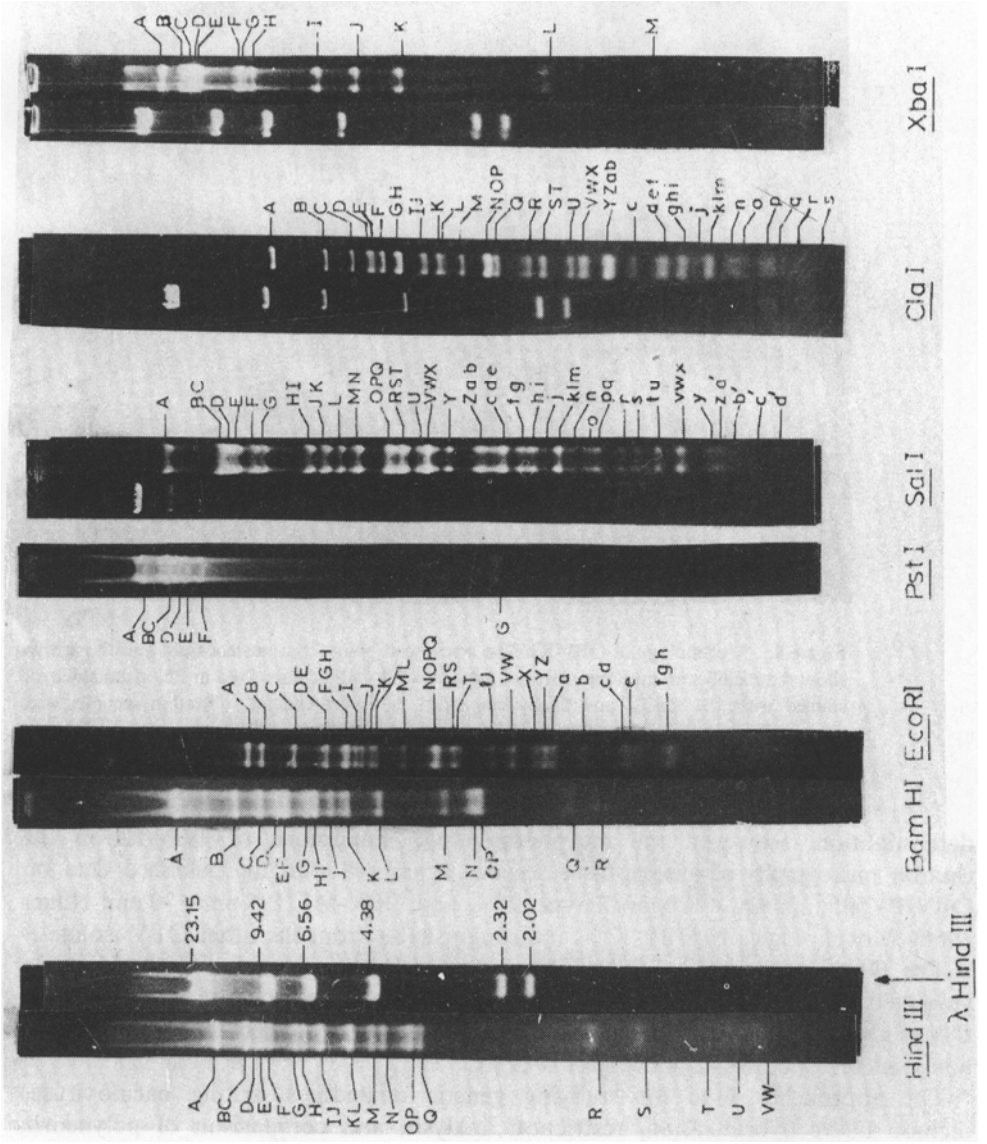
λ Hind III
Hind III
Bam HI
EcoRI
Pst I
Sal I
Cla I
Xba I
23.15
9.42
6.56
4.38
2.32
2.02

**Figure 2a.**

direct base-composition analysis, implying near-total homology between the two genomes.

Figure 2 a shows the restriction patterns of KI DNA cleaved by the restriction enzymes EcoRI (36 fragments), BamHI (19 fragments), HindIII (23 fragments), PstI (7 fragments), SalI (56 fragments), ClaI (45 fragments) and Xbal (13 fragments). Since

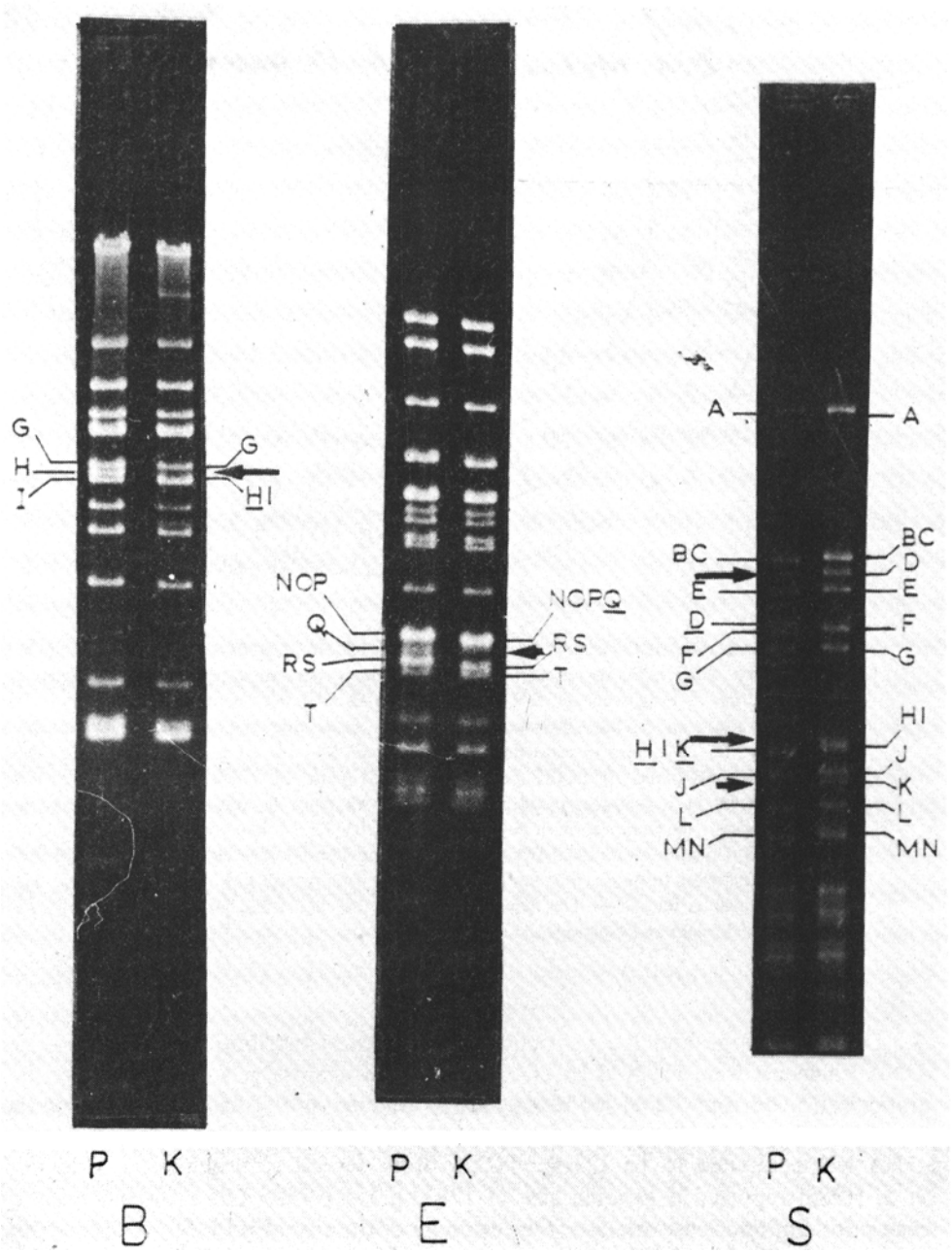


**Figure 2b.**

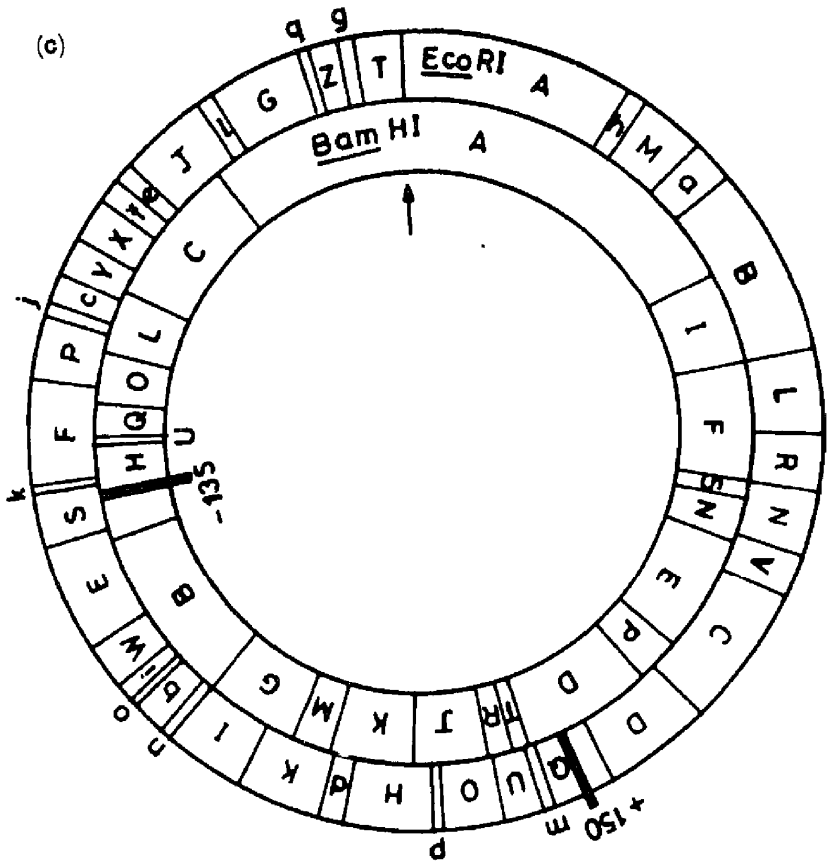


**Figure 2.** Restriction endonuclease analysis of KI DNA. **(a)** Purified KI DNA (2 $\mu$g) was digested with restriction enzyme and the fragments were separated in 0·8 % agarose gel at 2 V $cm^{-1}$. Fragments of λDNA digested with HindIII were used as size markers (in the SalI, ClaI and XbaI digestion panels the lanes on the left show the λDNA digest), **(b)** Differences in restriction profiles between KI (lane K) and PV505 (lane P) DNAs with BamHI (panel B), EcoRI (panel E) and SalI (panel S). The DNA fragments showing differences in mobility are underlined; the arrows point to regions in the gel showing differences, **(c)** Comparison of physical maps of OBV-KI (Mohan and Gopinathan 1991) and PV505 (Crawford *et al* 1985) DNAs for EcoRI and BamHI. The approximate locations of a DNA insertion ( + ) and a deletion (–) in KI DNA with respect to PV505 DNA, in base pairs, are shown as thick bars. The map shown here is the circularized form of the linear physical map of Mohan and Gopinathan (1991).

KI DNA was large and the restriction-fragment size distribution was varied, the DNA fragments were resolved by electrophoresis in agarose gels of different concentrations (0·5, 0·8 and 1·2%) for estimation of genome size. Table 1 summarizes the restriction-fragment size distribution of OBV-KI DNA. The average size of KI DNA was computed to be 123·04±1·0 kbp from several electrophoreses. Crawford *et al* (1985) reported a genome size of 126 kbp for PV505 DNA on the basis of restriction-endonuclease analysis. Our estimate of the size of KI DNA is also within the range for baculovirus genomes, which are known for their large sizes ranging from 60 to 250 kbp (Miller 1984).

**Table 1.** Sizes of restriction fragments of OBV-KI DNA.

| HindIII | | EcoRI | | BamHI | | PstI | | XbaI | | SalI | | ClaI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | S | F | | F | S | F | S | F | S | F | S | F | S |
| A | 15·37 | A | 10·69 | A | 33·68 | A | 35·91 | A | 33·00 | A | 11·05 | A | 9·39 |
| BC | 10·43* | B | 9·48 | B | 11·57 | B | 20·29 | B | 17·09 | BC | 5·90* | B | 6·61 |
| D | 9·48 | C | 7·25 | C | 9·06 | C | 19·49 | C | 12·64 | D | 5·63 | C | 5·68 |
| E | 9·06 | DE | 5·77* | D | 7·83 | D | 17·12 | D | 11·95 | E | 5·32 | D | 5·19 |
| F | 8·50 | FGH | 5·09† | EF | 7·25* | E | 15·36 | E | 11·33 | F | 4·72 | E | 5·06 |
| G | 6·73 | I | 4·79 | G | 6·28 | F | 13·18 | F | 8·05 | G | 4·44 | F | 4·81 |
| H | 6·39 | J | 4·65 | HI | 5·87* | G | 2·67 | G | 7·73 | HI | 3·36* | GH | 4·42* |
| IJ | 5·68* | K | 4·39 | J | 5·33 | | | H | 7·30 | J | 3·13 | IJ | 3·88* |
| KL | 4·65* | L | 4·27 | K | 4·79 | | | I | 4·85 | K | 3·05 | K | 3·63 |
| M | 4·10 | M | 3·73 | L | 3·99 | | | J | 4·01 | L | 2·87 | L | 3·55 |
| N | 3·88 | NOPQ | 3.17‡ | M | 2·85 | | | K | 3·20 | MN | 2·66* | M | 3·25 |
| OP | 3·33* | RS | 2·92 | N | 2·49 | | | L | 1·75 | OP | 2·34* | NOP | 2·90† |
| Q | 3·17 | T | 2·85 | OP | 2·41* | | | M | 1·19 | Q | 2·29 | Q | 2·78 |
| R | 1·52 | U | 2·43 | Q | 1·65 | | | | | RS | 2·22* | R | 2·41 |
| S | 1·27 | VW | 2·23* | R | 1·49 | | | | | T | 2·18 | ST | 2·28* |
| T | 1·08 | X | 2·03 | S | 0·99 | | | | | U | 2·07 | U | 2·03 |
| U | 1·01 | YZ | 1·97* | | | | | | | VWX | 2·01† | VWX | 1·92† |
| VW | 0·99* | a | 1·89 | | | | | | | Y | 1·85 | YZab | 1·75‡ |
| | | b | 1·70 | | | | | | | Zab | 1·61† | c | 1·59 |
| | | cd | 1·42* | | | | | | | cde | 1·48† | def | 1·44† |
| | | e | 1·38 | | | | | | | fg | 1·40* | ghi | 1·33† |
| | | fgh | 1·23† | | | | | | | hi | 1·33* | j | 1·25 |
| | | i | 0·96 | | | | | | | j | 1·28† | klm | 1·21† |
| | | j | 0·93 | | | | | | | klm | 1·23 | n | 1·12 |
| | | | | | | | | | | n | 1·17 | o | 1·07 |
| | | | | | | | | | | o | 1·12 | pq | 0·97* |
| | | | | | | | | | | pq | 1·09* | r | 0·88 |
| | | | | | | | | | | r | 1·01 | s | 0·76 |
| | | | | | | | | | | s | 0·96 | | |
| | | | | | | | | | | tu | 0·91* | | |
| | | | | | | | | | | vwx | 0·84† | | |
| | | | | | | | | | | y | 0·77 | | |
| | | | | | | | | | | za′ | 0·76* | | |
| | | | | | | | | | | b′ | 0·69 | | |
| | | | | | | | | | | c′ | 0·65 | | |
| | | | | | | | | | | d′ | 0·58 | | |
| Total | 121·72 | | 123·77 | | 123·03 | | 123·99 | | 124·06 | | 122·23 | | 117·56 |

F, Fragment; S, size (kbp).
*Doublet, †triplet, ‡quadruplet.
DNA fragment sizes were computed using the Sequaid program (Phamacia), with λDNA HindIII fragments as markers. For large and small DNA fragments, agarose gels of different percentage were used. The restriction fragments are designated A to Z, followed by a to z, and further a' to z', in decreasing size order.

There are clear differences in the restriction patterns of the DNAs of KI and PV505 with BamHI, EcoRI and SalI (figure 2 b), while the restriction patterns with PstI, HindIII, Xbal and ClaI are identical. The two DNAs can therefore be distinguished on the basis of differences in some restriction profiles. We have earlier constructed a linear physical map of OBV-KI DNA for the restriction enzymes BamHI, EcoRI, HindIII, PstI, SalI and XbaI (Mohan and Gopinathan 1991). In the present compareson between KI and PV505 DNAs, the few additional or missing fragments in one or the other of the juxtaposed restriction profiles (figure 2 c) were identified by estimation of the size differences between KI and PV505 DNAs (Mohan and Gopinathan 1991; Crawford *et al* 1985).

Crawford *et al* (1986) studied genotypic variations among 12 geographical isolates of OBV using restriction digestion, and noted insertions and deletions ( + 70, +70; – 100, –40 bp) in four regions in KI DNA with respect to the physical map of the DNA of the type isolate, PV505. We did not see these differences in the present studies, although the OBV-KI used by the former group was supplied by us. The restriction profiles we obtained of DNA from OBV-PV505 supplied by Crawford were identical with those reported by the latter's group. DNA insertions and deletions are reported to be of common occurrence in the genomes of baculoviruses propagated even in cell culture (Burand and Summers 1982; Miller and Miller 1982; Fraser *et al* 1983, 1985). In our studies, we propagated the virus in adult beetles.

The presence of genotypic variants among wild-type isolates of baculoviruses has been well documented (Mclntosh *et al* 1987; Smith and Crook 1988). These variants stem from recombination during mixed infections, acquisition of host-cell DNA, and mutations resulting in sequence deletion and reiteration within baculovirus genomes propagated *in vitro* or *in vivo*. Genetic recombination in OBV is postulated to be a rare occurrence in contrast to occluded baculoviruses, where most field isolates consist of a mixture of strains (Crook *et al* 1985). This is because of the labile nature of OBV, which does not allow OBV strains to accumulate and mix in the soil environment (Crawford and Zelazny 1990).

Comparison of SDS-PAGE profiles of proteins of KI and PV505 revealed differences (Mohan and Gopinathan 1989a). Nine and six isolate-specific proteins were attributed to PV505 and KI respectively. Differences were also noted between the isolates in glycosylation pattern of individual proteins. Antigenic heterogeneity between KI and PV505 has also been demonstrated using immunoblotting, and the difference has been quantitated using a modified ELISA (Mohan and Gopinathan 1989b).

The DNA sequence differences implied by alterations in restriction patterns and the differences in constituent proteins between KI and PV505 suggest the taxonomic status of a variant for OBV-KI.

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