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ANALYSIS OF THE GENOME OF PSEUDORABIES VIRUS ERCEGOVAC STRAIN WITH RESTRICTION ENDONUKLEASES

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The genome of pseudorabies virus Ercegovac vaccine strain was anlysed by the restriction enzymes Bam HI, Kpn I and Bgl II. The results of the autoradiograms show that the Ercegovac vaccine strain contains a genome that can be classified as a clas 3 DNA molecule. The analysis with Bam HI enzyme shows the loss of the Bam HI cleavage site between fragments 8 and 8'. The genome of Ercegovac strain has a deletion in the Us region.

Key words: Herpesvirus, Pseudorabies virus, endonucleases.

INTRODUCTION

Pseudorabies virus (PrV) or herpesvirus suis causes disease in swine through out much of the world, which results in several economic losses. The genome of PrV consists of a linear, double-standed DNA molecule approximately 150 kilobases in size. (Ben-Porat et al. 1984). Pseudorabies virus contains a class 2 DNA molecule, consisting of two segments, the unique long (Ul), and the unique short (Us). Segment the Us sequence is bracketed by inverted repeated sequences (IRS). As a consequence, the Us sequence inverts itself relative to the Ul sequence and two isomeric forms of the genome exist (Ben-Porat et al. 1979). The genome of the most attenuated vaccine strains contains an invertable Ul component as both the Ul and Us components are bracketed by inverted repeat sequences, and both components invert themselves relative to each other giving rise to four isomeric forms of the genome. Genomes with this structure have been designated class 3 DNA molecule.

The aim of the experiment described in this paper was to analyse the genome of Ercegovac vaccine strain with resriction endonucleases and to detect differences between the Ercegovac strain and the PrV (Ka) strain.

MATERIALS AND METHODS

Virus strain and cell culture

Prv (Ka) strain (Kaplan 1959) and Ercegovac vaccine strain (Ercegovac, 1960) viruses, were grown on pig kidney (PK-15) cells. Pig kidney PK-15 was

cultivated in Dulbecco synthetic medium containing 5% dialysed bovine serum. Eagle's minimal essential medium (MEM) was used for propagation of the viruses.

Enzymes and chemicals

α^{32} P d (CTP) was purchased from New England Nuclear Corp. Restriction enzymes were purchased from Bethesda Research Laboratories Inc.

Purification of viruses

Virions were purified as previously described by Ben-Porat et al. (1974).

Extration of DNA

Sodium sarcosinate (final concentration 2%) was added to the samples heated to 60^{0}C for 15 min. and digested with nuclease free pronase (1mg/ml) for 2 hours. The DNA was extracted four times with phenol-chloroform-isoamylalcohol (50:48:2vol/vol/vol) and alcohol precipitated (Ben-Porat et al. 1974).

Restriction enzyme digestion and gel electrophoresis of DNA fragments

Digestion and agarose gel electrophoresis of virus DNA were carried out as previously descibed by Rixon et al. (1979). Filter strips to which restriction fragments of PrV (DNA) were fixed, were prepared by the methods of Southern et al. (1975).

Nick translation of cloned PrV DNA restriction fragments

PrV DNA restriction fragments cloned in pBR 325 as described by Ladin et al. (1982), were nick translated by the method or Rigby et al. (1977)

RESULTS

The restriction patterns obtained by digestion of DNA of the prV (Ka) strain and the Ercegovac strain viruses with Bam HI and Kpn I enzymes are shown in Figure 1.

Several differences occurred between the restriction patterns of DNA of Ercegovac strain and of that of Ka strain. Some restriction fragments of the genome of Ercegovac strain had different migration rates. Variation in the migration patterns of some bands were observed especially in some hypervariable fragments which often vary in size when the genomes of differnet isolates are analysd (Lomniczi et al. 1984b, 1987b, Ben-Porat et al. 1984). The patterns generated by these enzymes from the genome of Ercegovac vaccine strain differed from those seen when the genome of other PrV strains were similarly analysed. To identify the nature of the differences between the restriction fragment patterns of the Ercegovac strain and of the wild-type PrV (Ka) strain, the genome of the Ercegovac strain was mapped by Southern technique using nick-translated cloned fragments of the PrV (Ka) as probes.

Figures 2 and 3 show hybridisation of the Bam HI cloned fragments of PrV (Ka) DNA to Bam HI and Kpn I digest of the Ercegovac strain genome. The autoradiograms illustrate the most important points.

The Bam HI and Kpn I maps of the Ercegovac strain as deduced from the hybridisation of Bam HI and Kpn I digests of all Bam HI restriction fragments of PrV (Ka) are illustrated in Figure 4. Restriction maps of the Ercegovac strain genome illustrate the inversion of the UI components.

On the basis of these results, it may be concluded that the Ercegovac strain contains a class 3 DNA molecule, probably as a consequence of translocation of

a sequence of nucleotides normally present at the end of the UI to the site next to the internal IRS.

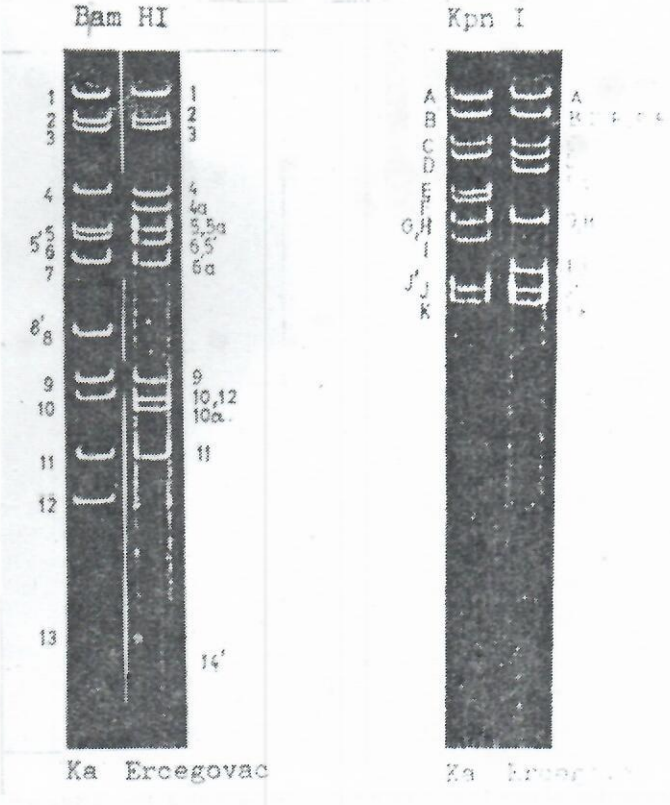

Figure 1. Restriction enzyme patterns of the DNA of PrV (Ka) strain and Ercegovac strain.

Details relevant for the construction of the physical map of the DNA of Ercegovac strain are described below. Cloned Bam HI fragment 14' hybridises normally to a single restriction fragment generated by digestion of standard viral DNA with either Bam HI or Kpn I digestion (Ben-Porat et al. 1979, Lomniczy et al. 1984b). The digestion of the Ercegovac strain genome with Bam HI yielded two fragments (fragments 5a and 14') and four fragments with Kpn I enzyme (fragments D/E and F/E comigrate, D and Fa) that hybridised to fragment 14'. The sequences that were translocated in the Ercegovac strain genome included, in addition to sequences derive from Bam HI 14', nearby sequences from Bam HI fragment 5' and included the Bam HI cleavage site. The sequences of the Bam HI fragments of Bam HI fragment 5' hybridised to two fragments in Bam HI digests (5' and 10a) Analysis of the data showed that the sequence of nucleotides (which is included in Bam HI fragments 14' and 5') normally present in the genome of

the PrV strains at the end of the UI sequence, is also present in the Ercegovac strain in the region next to the internal IRS (Figure 2).

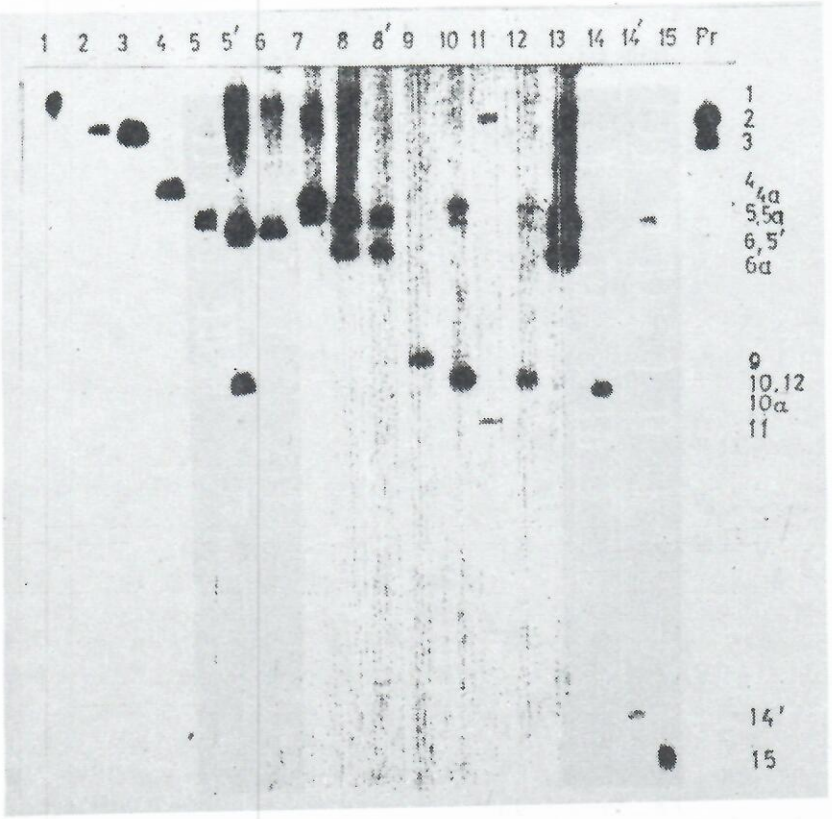

Figure 2. Hybridisation of Bam HI restriction fragments generated from Ercegovac strain genome to nick-translated, cloned Bam HI restriction fragments of PrV (Ka) DNA.

A finding of interest was that cloned Ba HI fragments 8,8' and 13 hybridise to two fragments in the Bam HI digestion of the DNA of Ercegovac strain. The fragments which hybridise to Bam HI fragments 8 and 8' and 13 were found in the position of fragments 5a and 6 (Figure 2). The fragment generated from the Ercegovac strain is a fusion fragment resulting from the loss of the Bam HI cleavage site between fragments 8 and 8'. The cloned Bam HI fragment 13 and fragment 8' hybridise to two fragments 5a and 6a generated by digestion of the Ercegovac strain with Bam HI enzyme (Figure 2). This result shows that fragment 13 is the same as fragment 8'. Because fragment 14' was found in position 5a in the Bam HI digestion, the loss of a cleavage site between fragments 8' and 14' was indicated.

The sequence that was translocated in the Ercegovac genome did not include fragment 8', as shown in restriction maps of the Ercegovac strain. Bam

HI fragment 8' hybridised to only three fragments in the Kpn I digestion (fragments D/E, F/E and H), but not to the Fa fragment (Figure 3) as occurred in the case of digestion of the genome of the MK-25 strain (Lomniczi et al. 1987b).

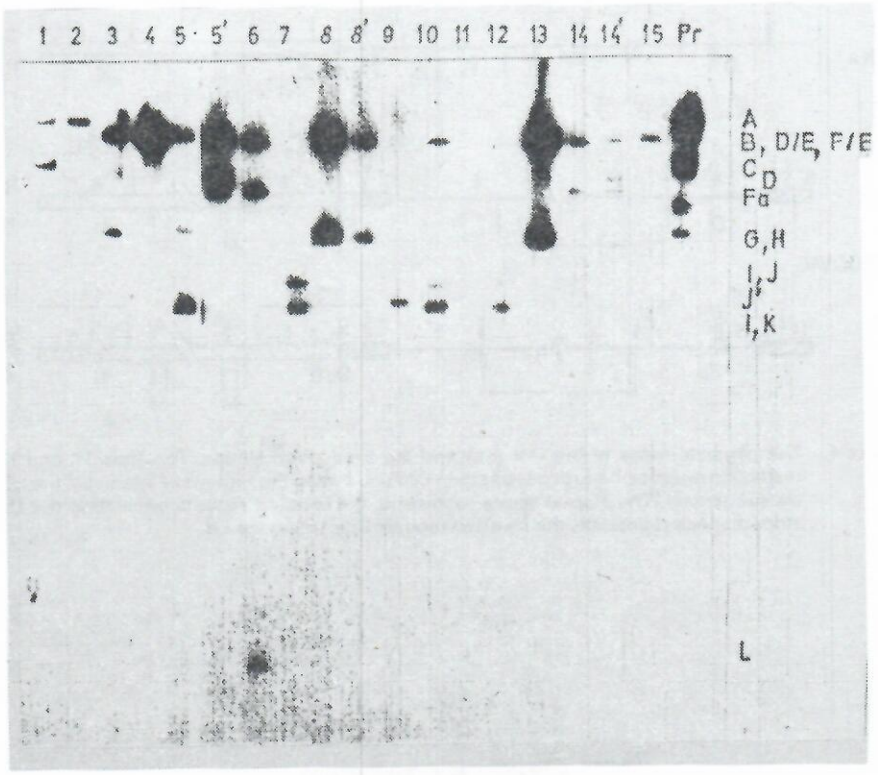

Figure 3. Hybridisation of Kpn I restriction fragments generated from Ercegovac strain genome to nick-translated, cloned Bam HI restriction fragments ofPrV (Ka) DNA.

Analysis of the genome of Ercegovac strain with Bgl II enzyme showed that the Ul inverted itself relative to Us. This is illustrated in Figures 5 and 6, which show the hybridisation pattern of the Bgl II digest of the Ercegovac and the PrV (Ka) strain to Bam HI fragments 14', 8', and 13. Fragment 14' hybridised to band A, Ba and C of the Ercegovac strain. Fragments 8' and 13 hybridised to bands A, D and E of the Ercegovac strain and not to band Ba which was generated as a result of the inversion of the Ul sequence. These results also confirm the conclusion that the genome of the Ercegovac strain contains a class 3 DNA molecule, but the sequences of the translocation do not include fragment 8' in Bam HI digestion.

The genome of the Ercegovac strain has a deletion in the sequence in the Us. The deletion was deduced from the Kpn I digestion patterns of the DNA

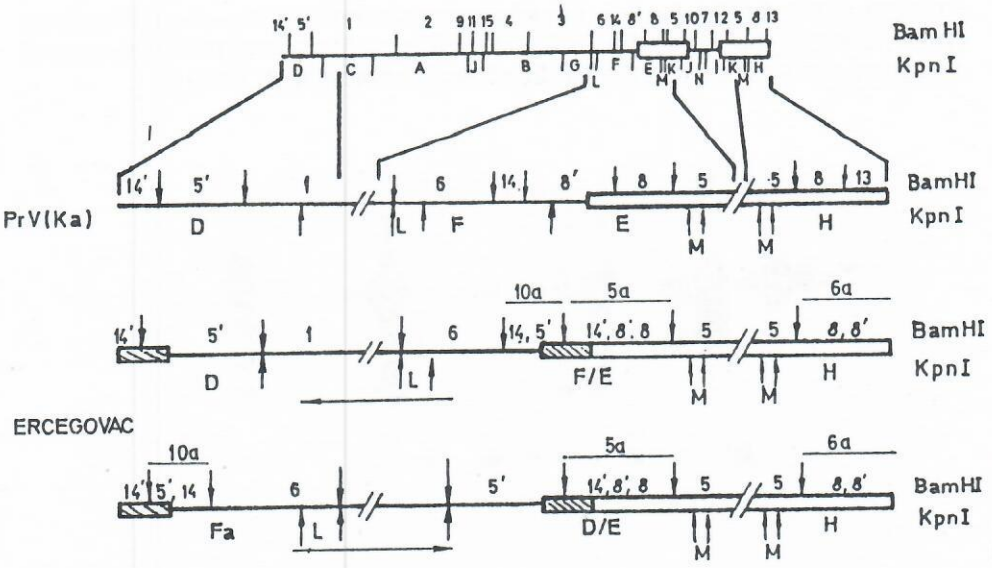

Figure 4. The physical maps of the PrV (Ka) and the Ercegovac strains. The Bam HI and Kpn I restriction maps of the Ercegovac strain DNA illustrated the regions of interest of the Ul and Us sequences. The striped space represents the inverted repeats bracketing the Ul: no striped space represents the inverted repeats bracketing the Us.

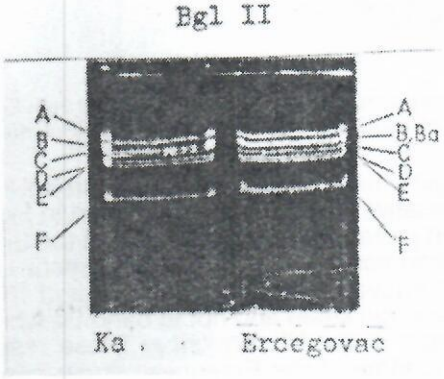

Figure 5. Restriction patterns with Bgl II enzyme of the DNA of the PrV (Ka) strain and the Ercegovac strain.

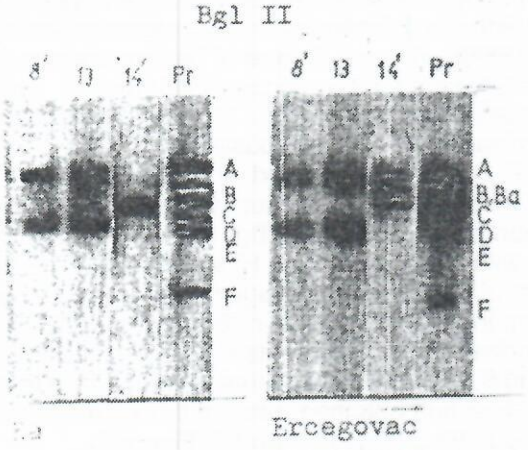

Figure 6. Hybridisation of Bgl II restriction fragments generated from the PrV (Ka) and Ercegovac strain genomes.

PrV(Ka) strain. Kpn I fragment I which included part of the Us was larger in the genome of wild-type virus than the Kpn I fragment I of the genome of Ercegovac strain (Figure 1 and Figure 2). Fragment 4a generated from the Ercegovac strain by Bam HI digestion which hybridised to Bam HI (7,10,12) was larger than the Bam HI fragment 7 generated from the PrV (Ka) strain (Figure 1), probably as a consequence of fusion of these fragments (Figure 2).

DISCUSSION

The Ercegovac strain was derived from a virulent strain that has been attenuated by more that 200 passages in chicken embryo fibroblasts. The original paternal PrV strain was isolated from an outbreak on a pig farm in Serbia. Several vaccine strains which have been passaged extensively in chicken embryo fibroblast cell cultures posses four isomeric forms and have a selective growth advantage over wild-type PrV when grown in avian cells (Lomniczi et al. 1987b).

The findings of this experiment show the Ercegovac vaccine strain contains a genome that can be classified as a class 3 herpesvirus DNA molecule Morover, the sequences that have been translocated in the Ercegovac strain genome did not include fragment 8'. A analysis with Bam HI ensyme showed loss of the cleavage site between fragments 8 and 8'. The genome of Ercegovac strain has a deletion in the sequence of the Us region.

The structure of the Ercegovac strain genome has been comperd with that of the genome of prV (Ka) as well as the Norden and MK-25 strains and some differences between them have been established. Analysis of the DNA of Ercegovac strain with restriction enzymes revealed that sequences derived from left and right of the genome have been translocated to the site to the IRS. That

the UI sequence is bracketd by inverted repeats is shown by the results of Bam HI and KpnI enzyme digests of 14' and 5' probes. Analysis of the genome of the Ercegovac strain with Bgl II confirmed this translocation, as in the Norden strain (Lomniczy et al. 1984b). A great degree of heterogenecity in the region of the repeats has been observed in the genome of other PrV which contain class 3 DNA molecules (Ben-Porat et al.1979b). Notable differences between the patterns of the Ercegovac strain and previously analysed Norden and MK-25 strains Lomniczi et al 1987. have been detected. The sequences that were translocated in the Ercegovac strain genome did not include fragment 8' as shown by the results of digestion using Kpn I and Bgl II enzyme. Also the analysis of the Ercegovac strain with the enzyme Bam HI showed fusion of fragments 8 and 8', resulting from loss of the Bam HI cleavage site between these fragments, which is normally present in the PrV (Ka) strain (Ben-Porat 1982). As a cloned Bam Hi framgnet 13 hybridises to two fragments in Bam HI digest in the same position as fragments 8 and 8' this data showed that fragment 13 is the same as fragment 8'. The results of digestion obtained with Bgl II confirmed the same. Fragments 13 and 8' hybridise to fragments A, D and E (Figure 6).

Several attenuated strains of PrV contain a genome which have a deletion in their short unique Us components (Lomniczi et al. 1984a). Both Batha and Norden strain have a deletion in the short Us region of the genome of approximately 2,7x10 daltons in size (Lomniczi et al. 1987a). All these strains are avirulent in swine and are either avirulent or have reduced virulence in a variety of laboratory animals. Despite the fact that the Ercegovac vaccine strain has been derived indepedently from a paternal PrV virulent strain, it also has a deletion in the Us region deduced from analysis with Kpn I enzyme. Many vaccine strains fail to express one or more glycoproteins (Gielkens et al. 1985). Thus, vaccinnated pigs do not develop an antibody response to the missing glycoprotein. This is important for eradication and control programs that are based on the serologic identification of swine infected with potently virulent strains from vaccinated swine (Mettenleiter et al. 1985, VAn Oirschot et al., 1987, 1988).

The results indicate that the genome of Ercegovac strain is a type 3 DNA molecule. The sequences that have been translocated did not include fragment 8'. The analysis with the enzyme Bam HI shows fusion of fragments 8 and 8' and has a deletion in the short unique sequence.

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ANALIZA GENOMA PSEUDORABIES VIRUSA SOJA ERCEGOVAC, RESTRIKCIONIM ENDONUKLEAZAMA

BEBA PANČIĆ

SADRŽAJ

Genom pseudorabies virusa vakcinalnog Ercegovac soja, analiziran je enzimima restrikcionim endonukleazama Bam HI, Kpn I i Bgl II. Rezultati autoradiograma pokazuju da Ercegovac soj sadrži genom tipa klase 3 DNA molekule. Analizom genoma sa BAm HI enzimom utvrđen je gubitak "mesta kidanja" Bam HI enzima između fragmenta 8 i 8'. Genom poseduje nedostatak sequenci nukleotida u Us regiji.