JOURNAL OF VIROLOGY, July 1983, p. 259–264
0022-538X/83/070259-06$02.00/0

Vol. 47, No. 1

Cloning and Cleavage Site Mapping of DNA from Bovine Herpesvirus 1 (Cooper Strain)

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Received 29 November 1982/Accepted 13 April 1983

Sequences representative of most of the bovine herpesvirus 1 (Cooper strain) DNA were cloned in the plasmid vector pBR322 at the *Hin*dIII site. *Eco*RI, *Hpa*I, and *Bam*HI restriction endonuclease sites were mapped in each of the cloned fragments, and this information was used to construct a restriction endonuclease cleavage site map of the entire viral genome for the four enzymes.

Bovine herpesvirus 1 (BHV-1), commonly known as infectious bovine rhinotracheitis virus, is a prominent cause of respiratory disease, abortion, conjunctivitis, and pustular vulvovaginitis in cattle (5). Less commonly, BHV-1 causes encephalitis and systemic infections in newborn calves.

Significant differences have been reported between the restriction endonuclease cleavage patterns of respiratory disease and pustular vulvovaginitis isolates of BHV-1 (3). In contrast, Misra et al. found, in their analysis of 116 BHV-1 isolates, that differences in cleavage patterns were not associated with different disease syndromes (V. Misra, L. A. Babiuk, and C. le Q. Darcel, Arch. Virol., in press). Three cleavage patterns were found among the 116 isolates, and on this basis the authors proposed three BHV-1 types which they designated strains I, II, and III.

A restriction endonuclease cleavage site map of BHV-1 strain K-22 has been determined by James Skare (personal communication; H. Ludwig, *Comprehensive Virology*, vol. 18, in press). The K-22 strain was isolated from a case of vulvovaginitis (6) and, based on restriction endonuclease analysis, it is classified as strain III (Misra et al., in press). In the present report, we give the restriction endonuclease cleavage site map of a respiratory disease isolate of BHV-1 (Cooper strain) and compare it with that of K-22. The Cooper strain is a respiratory disease isolate (9) and is used as the standard challenge strain for evaluating BHV-1 vaccines in the United States. It is of the strain I type.

Georgia bovine kidney cells (developed by R. F. Solarzano, Columbia, Mo.) were infected with virus at a multiplicity of 0.01 to 0.1 infectious particles per cell. At 100% cytopathic effect (generally 48 h postinfection), virus was harvested by centrifugation and purified by centrifugation in 10 to 40% potassium tartrate density gradients, according to published procedures (8). DNA was prepared by phenol extraction of 1% sodium dodecyl sulfate-disrupted virus followed by banding on CsCl density gradients.

Purified Cooper strain DNA was cleaved with restriction endonuclease *Hin*dIII, and the resulting fragments were cloned into the plasmid pBR322 with *Escherichia coli* strain HB101 as host by standard procedures (2). Plasmid DNAs from 92 ampicillin-resistant, tetracycline-sensitive colonies were isolated, cleaved with restriction endonuclease *Hin*dIII, and examined by gel electrophoresis for the presence of DNA fragments of the expected sizes. All of the plasmids examined contained DNA inserts, and all but seven of the inserts could be identified by size as particular BHV-1 *Hin*dIII digestion products. Examples are shown in Fig. 1. The molar fragment N and the one-half molar fragments, C, F, and H were not identified in our clone collection. The one-half molar fragment D was found only once, and all other fragments were identified a minimum of three times. The completed map (see Fig. 3) shows that fragments N, F, and H are ends and should not have been cloned by the technique used. The apparent difficulty in cloning fragments C and D is presumably related to the presence of repetitive sequences within these fragments (J. E. Farley, I. B. Skare, and J. Skare, Abstr. Int. Meet. Human Herpesviruses. *In* The Human Herpesviruses, 1980, p. 590). Perhaps it is significant that the single clone containing fragment D also contains fragment L, oriented so that the probable cloning event was the insertion of an L-D partial digest fragment into the plasmid. We have no explanation as to why an L-D fragment would be clonable whereas fragment D by itself would not be.

TABLE 1. Restriction endonuclease digestion fragment sizes of BHV-1 (Cooper strain) DNA[a]

*Hin*dIII		*Bam*HI			EcoRI			*Hpa*I		
Fragment	Measured size	Fragment	Measured size	Map size	Fragment	Measured size	Map size	Fragment	Measured size	Map size
A	21.4 (13.2)	(½)A	29.2	30.0 (18.5)	A	55	58.2 (35.9)	A	55	53.8 (32.9)
B	19.3 (11.9)	B	29.2	29.7 (18.3)	B	22.5	22.3 (13.8)	B	26.5	26.3 (16.2)
(½)C	15.9 (9.8)	(½)C	21.2	21.1 (13.0)	C	15.8	16.2 (9.7)	C	18.7	19.0 (11.7)
(½)D	14.8 (9.1)	(½)D	21.2	20.9 (12.9)	D	15.3	15.3 (9.4)	D	15.4	15.4 (9.5)
E	13.5 (8.3)	E	18.2	18.5 (11.4)	E	12.9	13.1 (8.0)	E	10.2	9.6 (5.9)
(½)F	12.8 (7.9)	F	17.3	17.4 (10.7)	F	8.4	8.5 (5.3)	F	8.7	8.6 (5.3)
G	11.9 (7.3)	G	15.7	15.6 (9.6)	G	2.9	3.1 (1.9)	G	4.0	4.0 (2.5)
(½)H	11.7 (7.2)[b]	H	12.3	12.2 (7.5)						
I	11.7 (7.2)	(½)I	12.3	12.0 (7.4)						
J	9.0 (5.6)	J	0.9	0.9 (0.6)						
K	8.4 (5.2)	K	0.25	0.35 (0.22)						
L	7.7 (4.8)									
M	3.6 (2.2)									
N	2.4 (1.5)									
O	0.36 (0.22)									

[a] Sizes are in kilobases, with megadaltons in parentheses.
[b] The size of *Hin*dIII-H was not directly measured.

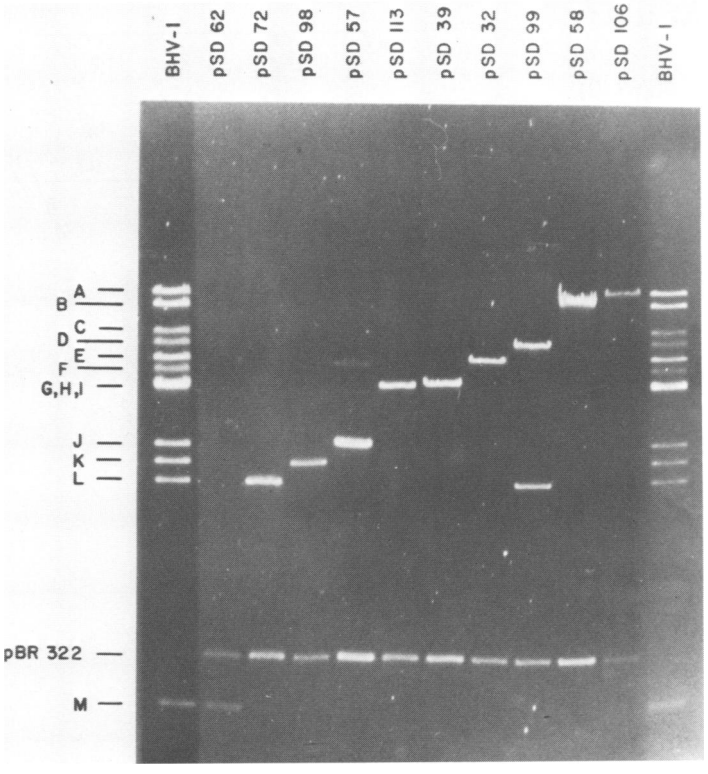

FIG. 1. A 0.5% agarose electrophoretic gel in 89 mM Tris–borate–3 mM EDTA (pH 8.3) stained with 1 μg of ethidium bromide per ml, demonstrating the *Hin*dIII digestion pattern of BHV-1 Cooper strain DNA and the corresponding BHV-1 cloned DNA fragments.

The size of each of the BHV-1 DNA *Hin*dIII digestion fragments was carefully determined by comparison with both *Hin*dIII and *Sal*I digestion fragments of phage lambda DNA. These fragment sizes are listed in Table 1. Cleavage maps of plasmids containing different cloned herpesvirus *Hin*dIII fragments were then prepared by measuring the fragment sizes resulting from appropriate single and double digests with the enzymes *Eco*RI, *Hpa*I, *Bam*HI, and *Hin*dIII. The plasmid maps are shown in Fig. 2. In all cases, the maps are unambiguous, and the internal agreement of the molecular weights is within 0.2 kilobases (kb).

Table 1 lists the restriction fragments produced from the Cooper strain of BHV-1 by the restriction endonucleases *Hin*dIII, *Eco*RI, *Hpa*I, and *Bam*HI. The correct map should predict these fragments, should not contradict the plasmid maps shown in Fig. 2, and should account for the three one-half molar fragments seen in the *Hin*dIII digest as well as the absence of visible one-half molar fragments in the other three digests. All of these requirements are met by the reconstruction shown in Fig. 3. The agreement between the measured and predicted fragments is generally excellent (Table 1).

Farley et al. (Abstr. Int. Meet. Human Herpesviruses) showed that BHV-1 strain K-22 DNA contains a terminal sequence of 7.5 megadaltons (Md) which is repeated internally in opposite polarity. This pair of inverted repeats and the intervening unique sequence are designated as the "S" region of the genome in Fig. 3. The presence of one-half molar digestion fragments and the absence of one-fourth molar fragments indicates that BHV-1 DNA occurs in two molecular forms, with the short unique sequence occurring in both orientations relative to the long unique sequence. This interpretation is confirmed by the mapping data summarized in Fig. 3. Thus, BHV-1 DNA is organized at this level in a very similar manner to pseudorabies virus DNA (1), equine herpesvirus 1 DNA (4), and varicella-zoster virus DNA (7).

Several features of the restriction endonuclease digestion patterns which are explained by the map deserve specific comment. First, a fourth one-half molar *Hin*dIII fragment is hidden in the large band labeled G,H,I in Fig. 1. We have assigned the name H to this fragment and have calculated its size to be 11.7 kb. With this assignment, *Hin*dIII fragment pairs C + H and D + F each total 27.6 kb. Second, the data of

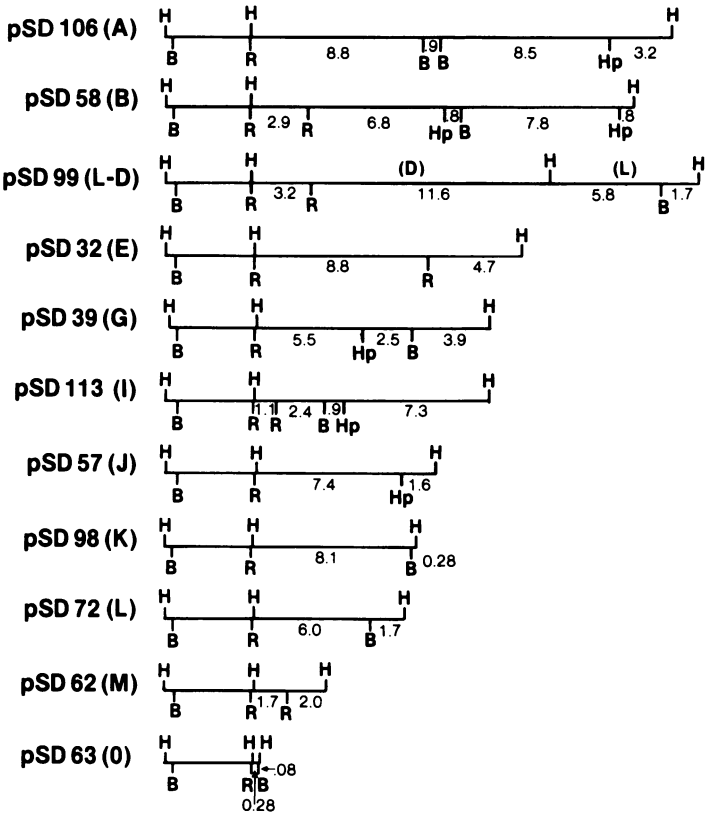

FIG. 2. Restriction endonuclease maps of recombinant plasmids, showing the location of the *Hin*dIII, *Eco*RI, *Hpa*I, and *Bam*HI sites. Abbreviations: H, *Hin*dIII; Hp, *Hpa*I; B, *Bam*HI; and R, *Eco*RI.

Farley et al. (Abstr. Int. Meet. Human Herpesviruses) require that the two *Eco*RI sites in the "S" region of the genome occur within the inverted repeat sequences. The absence of one-half molar *Eco*RI digestion fragments is consistent with this expectation. Third, the absence of easily identifiable one-half molar fragments from the *Bam*HI digest appears to be fortuitous. Each of the four one-half molar fragments produced by *Bam*HI digestion coelectrophoreses with another fragment. Thus, the *Bam*HI fragment pairs A and B, C and D, and H and I each form a single electrophoretic band on 0.5% agarose.

In general, our map confirms the previous work on strain K-22 (J. Skare, personal communication), although there are significant differences between the strains. There are 29 *Hin*dIII, *Eco*RI, and *Hpa*I sites in the two strains, and of these, 20 appear to be common to both. If we assume that the nine differences are due to single base pair changes, then the strains differ by about 5% in overall base sequence (9/174 = 0.05). In addition to this difference, the *Hin*dIII one-half molar fragments are each about 0.8 kb (0.5 Md) larger in strain K-22 than in Cooper strain. Because these fragments contain the terminal repeats flanking the "S" region (Farley et al. Abstr. Int. Meet. Human Herpesviruses), it is likely that the differences are due to additional repetitious DNA in this region of the K-22 genome. Thus, the data imply that the "S" region of the K-22 strain consists of two 12.2-kb (7.5-Md) inverted repeats flanking a 10.7-kb (6.6-Md) short unique sequence. In the Cooper strain, the short unique sequence is apparently about the same length as in strain K-22, but the inverted repeats are only about 11.4 kb (7.0 Md) in length.

Our calculated length for the total genome of 136.9 kb (84.5 Md) for Cooper strain and 138.5 kb (85.5 Md) for strain K-22 is slightly smaller than the 87.9 Md reported for strain K-22 (H. Ludwig, *in Comprehensive Virology*, vol. 18, in press).

These results provide a molecular basis for the future study of the virulent respiratory strains of infectious bovine rhinotracheitis virus and also provide quantitative data about the molecular

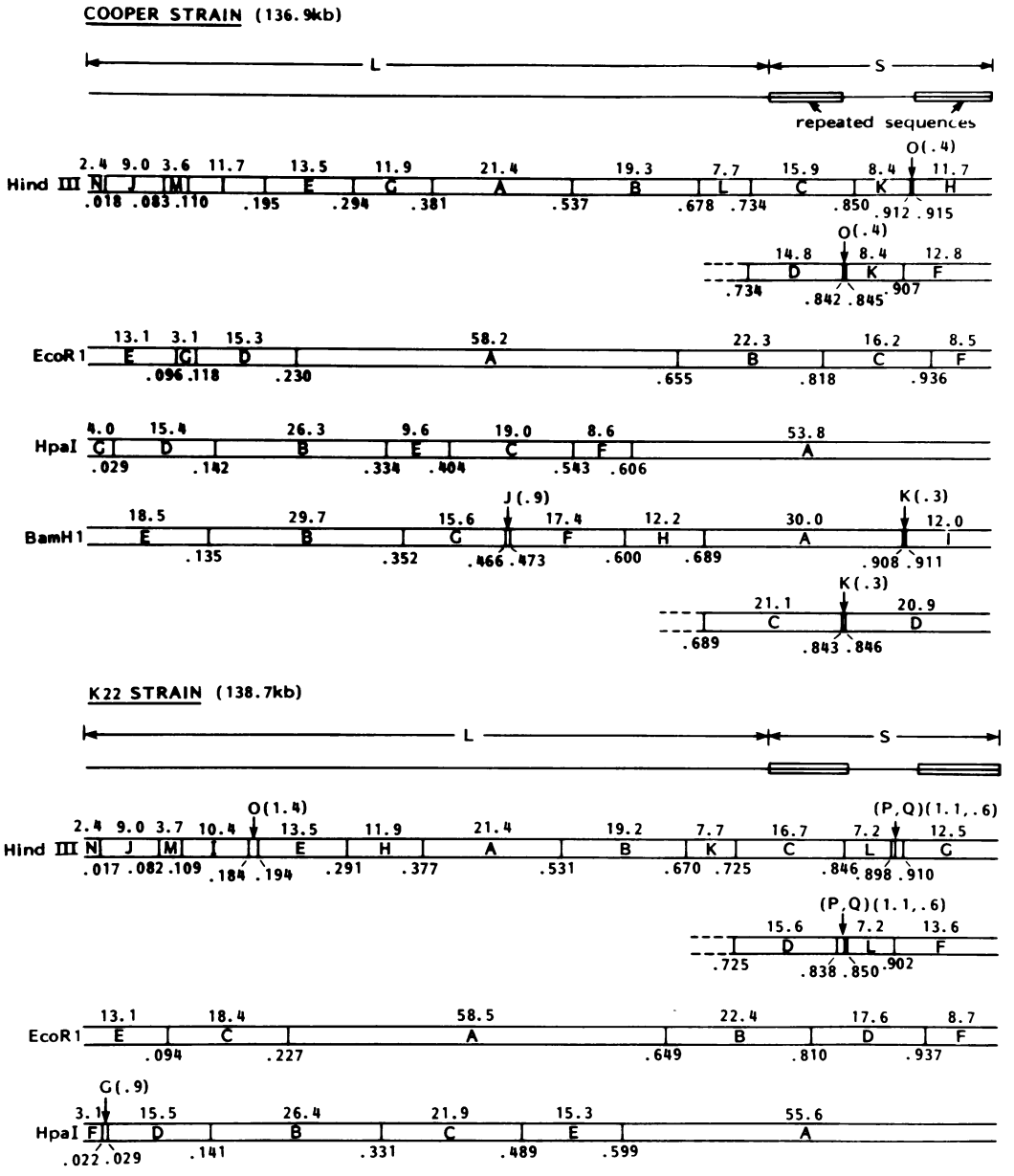

FIG. 3. Map of BHV-1 Cooper strain DNA compared with the map of BHV-1 strain K-22. Fragments are lettered according to size, with kilobases above each line and fractional map distance below. The approximate location of the tandem repeats is indicated. The map of strain K-22 was determined by James Skare (personal communication), but the distances between restriction sites have been adjusted slightly to be consistent with fragment size determination in our laboratory.

differences between strain I and strain III types of BHV-1 (Misra et al., in press).

We thank James Skare for making available his restriction endonuclease map of BHV-1 strain K-22 before publication. His data were first presented at the 1979 Cold Spring Harbor Herpesvirus Meeting. Although the data presented in this paper do not depend on his work, the availability of this map made our work substantially easier. The helpful assistance of Daniel Rock and of Dan Broderick was much appreciated.

This work was supported in part by an Iowa State University Internal research grant made possible by the Iowa State Alumni Achievement Foundation, and by U.S. Department of Agriculture competitive research grant 59-2191.

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