LETTER TO THE EDITOR

HOMOLOGY OF BOVINE HERPESVIRUS 2 AND HERPES SIMPLEX VIRUS 1 PROTEINS

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Bovine herpesvirus 2 (BHV-2, species *Bovine herpesvirus 2*, genus *Simplexvirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae*), has been known to have a close antigenic relationship with herpes simplex virus 1 (HSV-1) since antisera against BHV-2 or HSV-1 were reported to neutralize the growth of each of these viruses (*1*). Furthermore, inactivated HSV-1 or herpes simplex virus 2 (HSV-2), when inoculated into cattle, protects them against subsequent BHV-2 challenge (*2*). Prior BHV-2 infection also protects guinea pigs against genital HSV-2 infection (*3*). At the genome level, the structure of BHV-2 DNA is known to be similar to that of HSV-1 (*4, 5*). Furthermore, the sequences of thymidine kinase (TK) (UL23 gene), glycoprotein B (UL27 gene), ICP18.5 (UL28 gene), ICP8 (UL29 gene), DNA polymerase (UL30 gene), glycoprotein G (US4 gene), large and small ribonucleotide reductase subunits (UL39 and UL40 genes, respectively) show homology to HSV-1 (*6, 7, 8*). Based on the sequences of four genes (UL23 and UL27-UL29, determined over a decade ago (*6,9*) and serology and genome structure data, BHV-2 was classified in the species *Bovine herpesvirus 2, Simplexvirus* along with HSV-1 and HSV-2 and some primate viruses. As the total number of BHV-2 genes sequenced thus far is only eight out of at least 70, we report here additional sequences that double the number of currently completely sequenced BHV-2 genes spanning the whole unique long (UL) region of the BHV-2 genome (*4*).

BHV-2 DNA was prepared and digested with restriction nucleases and the obtained fragments were cloned into pUC18 plasmids. The recombinant plasmids were purified (*9*). The cloned *Hind*III or *Xba*I fragments were characterized relative to the size and designations published earlier (*5, 9*). DNA of BHV-2 strain BHM-1 was used for the sequencing of part of *Hind*IIII-I (UL20, UL21 and UL22 genes), *Xba*I-H (UL22 gene), *Hind*III-J (UL41 gene) and *Hind*III-O (UL8 gene) fragments. The genome location of these fragments is known (*5*). Subcloned regions of the *Hind*III-I fragment were previously described (*9*), when used in the localization of the TK gene. Cloned *Sal*I fragments of DNA of BHV-2 strain C290 (*3*) were used to sequence UL3 and UL4 (both of 3.3 K) as well as UL55 (3.5 K) genes; however, the genome location of these fragments remained undetermined. The sequencing was performed at the Monash University Sequencing Facility, (Melbourne, Australia), using a sequencing kit (the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit employing Amplitaq DNA polymerase) and an automatic sequencer (the AB373 DNA Sequencer-strech). Standard M13pUC forward and reverse oligonucleotide primers were used, followed by primers designed on the basis of the sequences obtained. The sequencing was done at least in triplicate. The sequences

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Abbreviations: BHV-2 = bovine herpesvirus 2, EHV-1 = equine herpesvirus 1; HSV-1 = herpes simplex virus 1; HSV-2 = herpes simplex virus 2; BHV-1 = bovine herpesvirus 1, MDV-2 = Marek's disease virus 2; PrV = pseudorabies virus, SVV = simian varicella virus; TK = thymidine kinase; UL = unique long; VZV = varicella-zoster virus

Gene	No of aa	M_r (K)	Amino acid homology (%)								
			BHV-2	HSV-2	HSV-1	VZV	EHV-1	BHV-1	SVV	MDV-2	PrV
UL3	213	22 3	100	58	55	56	49	52	47	49	38
UL4	210	22 1	100	43	44	33	24	29	31	33	23
UL8	733	78.6	100	37	36	26	26	27	24	21	27
UL20	222	24.2	100	55	57	23	24	27	22	26	34
UL21	522	56 0	100	49	49	29	33	29	32	25	30
UL22	867	94 0	100	44	46	26	24	28	22	21	28
UL41	487	54 8	100	59[a]	59	39	35	34	34	30	43
UL55	193	21 3	100	44	45	28	29	NIL	25	28	NIL

For abbreviations of virus names see the list of abbreviations at the front page of the article
[a]Identical value was found for the baboon herpesvirus
M_r = relative molecular mass of the protein
NIL = no equivalent gene

were monitored using the NCBI Blast System as well as the DNA Strider™ 1.2 program. Final nucleotide sequences were submitted to the Genebank. The Acc. Nos. given to the variety of BHV-2 genes are as follows: AF383175 for the UL3 nucleophosphoprotein gene, AF383176 for the UL4 nuclear protein, AF372518 for the UL8 helicase/primase-associated protein gene, AY027921 for the UL20 integral membrane protein gene, AF387490 for the UL21 tegument protein gene, AF375976 for the UL22 glycoprotein H gene, AY033933 for the UL41host shutoff protein, and AF383177 for the UL55 protein gene.

The predicted protein products of UL3 (*10*), UL4 (*11*), UL8 (*12*), UL20 (*13*), UL21 (*13*), UL22 (*14*), UL41 (*15*), and UL55 (*16*) genes of alphaherpesviruses (members of the subfamily of *Alphaherpesvirinae)* had all been extensively compared elsewhere (see the indicated references). The homology of the deduced amino acids of the sequenced BHV-2 genes and those of other herpesviruses is shown in the table. The predicted size of each of the BHV-2 proteins is also indicated. All BHV-2 gene products show the greatest homology to HSV-1 and HSV-2. However, the UL41 gene, one of the few sequenced genes of the baboon herpesvirus 2 *(17)*, had the same homology with the corresponding BHV-2 gene at the amino acid level as had HSV-1 and HSV-2. This result further confirms a close relationship between genes and their products of some primate herpesviruses (simian agent 8 and B virus) and BHV-2 (*6, 8*). In contrast, the simian varicella virus (SVV) gene products had much lower homology with those of BHV-2 (see the table) or other animal viruses. The nucleotide sequencing also indicated that no UL3.5 gene exists in the BHV-2 genome, in which only 51 nucleotides separate UL3 and UL4 genes. The size of the UL3.5 gene product can range from 71 (varicella-zoster virus, VZV) to 224 (pseudorabies virus, PrV) amino acids *(18)*. The lack of this gene is also a feature of the HSV-1 and HSV-2 genomes. However, the UL3.5 gene is present in VZV, EHV-1, PrV, BHV-1 and infectious laryngotacheitis virus *(18)*. Moreover, the BHV-2 genome contains a UL55 gene, which is absent in PrV and BHV-1 *(19)*.

From the known BHV-2 genome structure (*4*) and the *Hind*III and *Xba*I restriction fragments locations (*5, 9*) the BHV-2 genes described here appear to be located at positions within the UL region similarly to the HSV-1 genome. The size of UL regions of HSV-1 and BHV-2 (excluding the inverted terminal repeat regions) is almost identical (100 K) (*4*). The HSV-1 UL region contains the genes UL1- UL56 *(20)*; the number of genes in the BHV-2 UL region is unknown. During this study several other partial gene sequences were generated which also showed direction of translation of neighbour genes and indicated that the completely sequenced genes described here are oriented in a manner identical with that of the corresponding HSV-1 genes *(20)*. The sequencing of the genes spanning the UL region of BHV-2 genome indicated that all these genes have a close relationship to HSV-1 and HSV-2, at least at the amino acid level, and suggested that the homology is not restricted to some particular region of the genome. This further confirms that BHV-2 may have diverged from a common ancestor to HSV-1 (*6, 8*).

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