Indian Journal of Experimental Biology
Vol. 34, November 1996 pp. 1169-1171

Direct detection of bovine herpes virus-1 DNA from cell culture fluids using polymerase chain reaction

B P Sreenivasa, T J Rasool* & C Natarajan
Indian Veterinary Research Institute, Bangalore 560 024, India

Received 28 September 1995; revised 21 May 1996

A pair of oligomers of 20 and 23 bp were designed for amplifying a 381 bp sequence from glycoprotein IV gene of bovine herpesvirus 1. The primer pairs were used for amplifying genomic DNA of BHV-1 directly from cell culture fluids under different experimental conditions such as, untreated cell culture fluid, thermal denaturation and proteinase K treatment in presence of detergent . The results reveal that direct thermal denaturation of cell culture fluid is sufficient to detect the virus by polymerase chain reaction.

Bovine herpesvirus-1 (BHV-1) is an economically important pathogen of cattle responsible for various diseases like infectious bovine rhinotracheitis (IBR), abortions, conjunctivitis, and infectious pustular vulvovaginitis[1]. Cattle infected with BHV-1 usually survive infection but the virus persists in latent form. The latently infected cattle serve as virus carriers, ensuring the perpetuation of the virus in cattle population. The cross breeding programme followed in Indian subcontinent using exotic bulls/germ plasm helps rapid dissemination of virus and hence a convenient and sensitive diagnostic technique is highly desirable for screening all the suspected carriers of the virus, especially the breeding bulls.

Diagnosis of BHV-1 infection has usually been done by serology, virus isolation, detection of viral antigens by enzyme linked immunosorbent assay, immunofluorescence[2] and by nucleic acid hybridization technique[3-5]. However, these techniques are time consuming and less sensitive. Application of PCR technique[6], one of the most frequently employed diagnostic technique for detection of virus[7] has also been extended to BHV-1 detection[8-10] inspite of the problems associated with high GC content in virus genome. All the PCR based detection of BHV-1, so far reported, involved isolation of viral genomic DNA[8-10]. In this communication, a rapid detection method using PCR directly from cell culture fluids circumventing DNA isolation is reported.

One primer pair of 20 and 23 nucleotides was designed using standard primer selection criteria[11] based on the published sequence information for glycoprotein IV (gIV) gene[12] and synthesized from Microsynth, Switzerland. A longer sequence (381 bp) was included between the primers so that the fragment can be used as probe for hybridization studies. Care was also taken to include restriction endonuclease cleavage sites for enzymes with potential for detecting variation, such as, HaeIII, TaqI, AluI, etc., so as to initiate PCR-restriction length polymorphism studies. The upstream primer is P1-5'-dACATGCTG-GCGLTGATCGLA-OH-3' (corresponding to positions +227 to +246) and the downstream primer is P2-5'-dCGAGTTTCGAGAACCAGCAGTCC-OH-3' (complementary to positions +585 to +607).

PCR was carried out with differential treatment of infected cell culture fluids. In one set of experiment, 5µl virus supernatant (titre $10^{7.5}$ $\log_{10}$ $TCID_{50}$/ml) was heated for 15 min, at 95°C, snap cooled and centrifuged for removing coagulated proteins. In another set of experiment, the virus supernatant was used as such without any pretreatment. In the third experiment, the supernatant was incubated with proteinase K (200 µg/ml) and SDS (0.5%) at 55°C for 30 min, followed by repeated phenol chloroform extraction and alcohol precipitation of DNA. The precipitate was dissolved in 10 µl of distilled water after drying. The PCR mix contained 5 µl of the above viral fluid/DNA, 200µM dNTPs, 10 pmols of each primer,

*Correspondent author

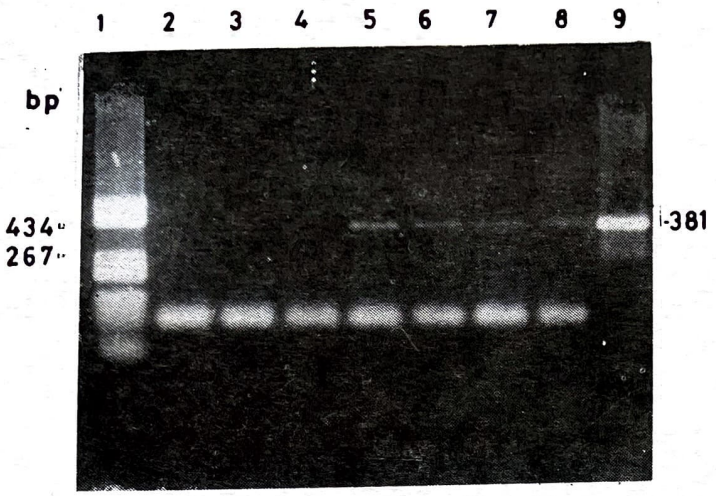

Fig. 1—PCR amplification of selected gD sequence of BHV-1 genome from cell culture fluids. [Lane 1: Marker pBR322 HaeIII digest, 2 : Negative control, 3 and 4 : Cell culture fluid without pretreatment, 5 and 6 : Cell culture fluid with thermal denaturation, 7 and 8 : Cell culture fluid with SDS (0.5%) and proteinase K (0.2mg/ml) treatment and 9 : Positive control with purified BHV-1 DNA].

2.5µl of 10 × reaction buffer and 0.5 units of *Taq* DNA polymerase (Bangalore Genie) in a total reaction volume of 25 µl. All the samples were heated for 5min. at 95°C and snap cooled before setting the PCR. Amplification was carried out initially for 5 cycles at 95°C for 1 min, 57°C for 2 min and 72°C for 1 min followed by 30 cycles at 95°C for 40 sec, 57°C for 40 sec and 72°C for 40 sec in a Perkin-Elmer-Cetus DNA thermal cycler. The PCR product was resolved in 1.5% agarose gel and examined using UV transilluminator after staining with ethidium bromide.

PCR products obtained with the primer pairs from differentially treated cell culture fluids are shown in Fig.1. Lanes 3 and 4 represent cell culture fluid used without any pretreatment, which apparently did not amplify any product. It may be due to the lack of naked DNA, even though the presence of virus in the supernatant was confirmed by infectivity in cell culture. Lanes 5 and 6 represent thermally denatured samples which gave a clear band of 381 bp. The supernatant which was pretreated with proteinase K in the presence of SDS resulted in weak amplification (lanes 7 and 8), may be due to low concentration of initial template DNA. It appears to be due to the loss of target DNA during the isolation procedure or inhibitory effects of the reagents used for DNA isolation on PCR. It is worth mentioning that only 5µl supernatant was heat treated and used for amplification, whereas 25-fold excess of the supernatant was used for isolating the DNA. Lane 9 represents amplification product from pure DNA isolated from purified virion which gave an intense band of 381 bp. Lane 2 was the negative control (distilled water) where no band was present clearly showing lack of any PCR contamination.

The possibility of related virus/viruses getting amplified with the same primer sequences cannot be ruled out as it has been shown that a high degree of sequence conservation exists among different ruminant herpesviruses[13,14]. However, this problem can be obviated by employing the restriction enzyme analysis of the PCR product.

The present study shows that PCR technique using thermally denatured viral supernatant could be used as a corollary test along with the isolation of virus in cell culture system for diagnosis of BHV-1 infection. The usefulness of this technique needs to be evaluated by employing it on field samples such as, nasal and ocular discharges, preputial washings, etc. from suspected cases of BHV-1 infections. Since direct PCR (without prior DNA extraction) for diagnosis of viral diseases has been reported on body fluids such as urine, cerebrospinal fluid, and pleural or peritoneal effusions[15,16], it may be possible to detect BHV-1 also directly from the body fluids by the above method.

References

1 Gibbs E P J & Rweyemamu M M, *Vet Bull*, 47 (1977) 317.
2 Van Donkersgoed J & Babiuk L A, *Vet Med*, 1 (1991) 86.
3 Dorman M A, Blair C D, Collins J K & Beaty B J, *J Clin Microbiol*, 22 (1985) 990.
4 Rock D L, Hagemoser W A, Osorio F A & Reed D E, *J Gen Virol*, 67 (1986) 2515.
5 Belak S, Linne T, Magyar G, Harrach B, Benko M, Klingeborn B, Klintevall K & Bartha A, *Mol Cell Probe*, 2 (1988) 147.
6 Mullis K B & Faloona F A, *Methods Enzymol*, 155(1987) 3335.
7 Lynch J R & Brown J M, *J Med Genet*, 27 (1990)2.
8 Wiedmann M, Brandon R, Wagner P, Dubovi E J & Batt C A, *J Virol Methods*, 44 (1993) 129.
9 Vilcek S, Nettleton P F, Herring J A & Herring A J, *Vet Microbiol*, 42 (1994) 53.
10 Van Engelenburg F A C, Van Schie F W, Rijsewijk F A M & Van Oirschot J T, *J Clin Microbiol*, 33 (1995) 308.
11 Rappolee D A, *Amplification: A forum for PCR users*, Issue 4 (The Perkin-Elmer Corporation, Applied Biosystems Division, Foster City, USA.) 1993, 5.
12 Tikoo S K, Fitzpatrick D R, Babiuk L A & Zamb T J, *J Virol*, 64 (1990) 5132.

13 Engels M, Loepfe E, Wild P, Schraner E & Wyler R, *J Gen Virol*, 68 (1987) 2019.

14 Vanderplaschen A, Bublot M, Pastoret P -P & Thiry E, *Arch Virol*, 128 (1993) 379.

15 Young L S, Bevan I S & Johnson M A, *Br Med J*, 298 (1989) 14.

16 Demmler G J, Buffone G J, Schimbor C M & May R A, *J Infect Dis*, 158 (1988) 1177.