Virus Research 158 (2011) 277–280

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Sequence analysis of topoisomerase gene of pseudocowpoxvirus isolates from camels (*Camelus dromedarius*)

G. Nagarajan*, Shelesh Kumar Swami, Shyam Singh Dahiya, G. Sivakumar, Shirish Dadarao Narnaware, F.C. Tuteja, N.V. Patil

National Research Centre on Camel, Post Bag No. 7, Jorbeer, Bikaner 334 001, Rajasthan, India

ARTICLE INFO

Article history:
Received 21 January 2011
Received in revised form 20 March 2011
Accepted 22 March 2011
Available online 8 April 2011

Keywords:
Pseudocowpoxvirus
Topoisomerase
PCR
Camel

ABSTRACT

Topoisomerase gene of pseudocowposvirus from Indian dromedarian camel was amplified by PCR using the primers of PCPV from Finnish reindeer and cloned into pGEM-T for sequence analysis. Analysis of amino acid identity revealed that Indian PCPV of camel shared 95.9–96.8 with PCPV of reindeer, 96.2–96.5 with ORFV and 87.5 with BPSV.

Poxviruses are large DNA viruses containing a double stranded DNA genome with closed hairpin loop ends. The family Poxviridae is divided into Entomopoxvirinae (insect poxviruses) and Chordopoxvirinae (vertebrate poxviruses) subfamilies; and the latter is further divided into eight genera, including parapoxviruses (PPV) (Murphy et al., 1999). Genus parapoxvirus has four members, orf virus (ORFV), bovine papular stomatitis virus (BPSV), pseudocowposvirus (PCPV), and parapoxvirus of red deer in New Zealand (PVNZ) (Hosamani et al., 2006; Inoshima et al., 2000). Parapoxviruses are distinguished from other pox virus by their high G + C content (approximately 64%) (Moss, 2001; Delhon et al., 2004). The viral genome is composed of ~135 kb linear, ds DNA with closed hairpin loop ends and genes located on both strands with a bi-directional orientation. Conserved genes are found in the central region of the genome, while variability is observed in the terminal ends (Mercer et al., 2002).

Pox and pox-like diseases of camels are a group of exanthematous skin conditions that have become increasingly important economically. They may be caused by three distinct viruses: Orthopoxvirus cameli (camelpox), papillomavirus (camel papillomatosis) and parapoxvirus (camel contagious ecthyma) (Munz, 1992). Camelpox is a highly contagious viral disease affecting mostly young animals and is characterized by popular pustular eruptions on the skin and mucous membranes. On the other hand, papillomatosis in camels leads to a wartlike lesion often found around the lips and nostrils and may be misdiagnosed as a pox disease, especially where generalized lesions occur.

Contagious ecthyma in humans and ruminants is caused by Orf virus (Fauquet et al., 2005) while in cattle the disease is caused by pseudocowpox (PCPV) and bovine papular stomatitis virus (BPSV) (Moyer et al., 2000). The disease camel contagious ecthyma (CCE) is widely recognized in camel-rearing regions of the world (Ali et al., 1991; Housawi et al., 2004). Recently, pseudocowposvirus (PCPV) has been reported as the etiological agent of CCE (Abubakr et al., 2007; Nagarajan et al., 2010). In CCE, nodules appeared on the lips of affected animals followed in most cases with swelling of the face and sometimes the neck. Papules and vesicles appeared later and within a few days developed into thick scabs. Lesions occurred sometimes on the face, eyes and nares. Healing occurred within 20–30 days in most cases (Khalafalla, 1998).

Topoisomerase enzymes release DNA supertwists and are required to facilitate transcription, DNA replication, and DNA repair processes (Kornberg and Baker, 1991; Wang, 2002). DNA topoisomerases are grouped into two families: type I enzymes break one DNA strand at a time, whereas type II enzymes break both strands simultaneously. All poxviruses encode an unusual type IB topoisomerase, which is a member of a family that is now known to include enzymes from mimivirus and many bacteria (Benarroch et al., 2006; Gubser et al., 2004; Upton et al., 2003). At about 33 kDa in size, these are by far the smallest topoisomerases known. Purified enzymes have been studied from vaccinia, variola virus, molluscum contagiosum (MCV), leporipox (Palaniyar et al., 1996), and two entomopox viruses (MSV and AMV) (Bauer et al., 1977; Krogh et al., 1999; Perry et al., 2006; Petersen et al., 1997; Shuman, 1998).

* Corresponding author. Tel.: +91 151 2230183; fax: +91 151 2231213.
E-mail address: camelnag@yahoo.com (G. Nagarajan).

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.03.020

The vaccinia virus topoisomerase can resolve Holliday junctions (HJs) *in vitro*, suggesting a role in the resolution of viral DNA concatemers to form unit genomes with hairpin telomeres (Shuman, 1998; Palaniyar et al., 1999; Sekiguchi et al., 1996). Knocking out of the vaccinia virus topoisomerase gene leads to low infectivity, which correlated with reduced early transcription rather than direct effects on the replication or processing of viral DNA (Da Fonseca and Moss, 2003). In addition, topoisomerases are excellent targets for anticancer and antimicrobial drugs. Further research on topoisomerase gene has importance in view to develop topoisomerase inhibitors as poxvirus therapeutics.

The objective of the present study was to amplify the topoisomerase gene of pseudocowposvirus isolates from Indian dromedarian camels by PCR and subsequent cloning of the PCR amplified DNA fragment into the vector for sequence analysis and to find out its relatedness with the other parapoxviruses available in the NCBI database.

In the mid September 2010, camel calves of below one year of age of either sex in the camel herd of NRC on Camel, Bikaner, Rajasthan, India were showing symptoms of contagious ecthyma lesions around the facial region. Scab materials were collected from a total number of 15 severely affected animals. Total DNA was extracted from collected skin scabs using AxyPrep Multisource Genomic DNA Miniprep kit (Geneaxy Scientific Pvt. Ltd.) according to the manufacturer's instructions. Nucleotide primers were designed using the sequence of the topoisomerase gene of the pseudocowpoxvirus isolate from Finnish reindeer (GenBank accession No. GQ329669); forward primer (TopoF) (5′-ACGAGGATCCATGCGGGCGCTGCACCTATCCGAC-3′), reverse primer (TopoR) (5′-TAGCGAATTCTCATCCATTTACCTGCTCCGAG-3′). PCR amplification of the topoisomerase gene was performed using the following thermal profiles: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR-amplified products were checked by electrophoresis in a 1% agarose gel. After purification of the amplified products from the low melting point agarose gel by phenol extraction followed by ethanol precipitation, the fragment was cloned in pGEM-T Easy Vector (Promega). The ligated mixture was used for transformation into *Escherichia coli* DH 5α (Sambrook et al., 1989). Positive clones were identified by colony PCR using gene-specific primers and restriction analysis with EcoRI. Positive clones were sequenced in both directions using universal T7 and SP6 primers at the DNA sequencing facility of Delhi University (South Campus), Delhi and analyzed with that of seven parapoxviruses published earlier in the GenBank (Table 1) using computer software BIOEDIT Version 7.0.9. These sequences were compared in Clustal X (Thompson et al., 1997) and phylogenetic tree was constructed in Treeview 1.6.5 by neighbour joining method (Page, 1996). The open reading frame (ORF) and motifs such as asn-glycosylation, myristylation and amidation of the gene products were predicted by the use of program GENERUNNER (hastings Software Inc., Hastings, NY, USA; http://www.generunner.net)

The disease was characterized by papules and then pustules on the lips-muzzle and eye lids of infected camels. Profuse salivation, foul mouth odour and facial edema were also observed. The pustules on the lips ruptured and became ulcerated. Those in the muzzle dried and became covered by grey or brown scabs (Fig. 1). Infected animals were showing pruritis and intermittent rubbing against the wall of the corrals, which eventually led to the sloughing of the skin at the affected areas.

Total DNA was extracted from all the 15 infected scabs and topoisomerase gene was amplified. DNA fragment of topoisomerase gene (957 bp) was observed on agarose gel electrophoresis. There was no amplification in the PCR using the DNA isolated from the camelpox positive scab materials (negative control). The clinical signs of camelpox, camel contagious ecthyma and camel papillomatosis are similar and can be confused, especially in the generalized form (Munz et al., 1990) and so far can be distinguished only by virus identification in electron microscope. In addition to the complexity and high skills required to operate electron microscopy, this technique is not usually available for veterinarians in the field services. Therefore, with the advent of molecular tools such as PCR and gene sequencing, it is possible to detect even a few copies of viral DNA from the clinical samples. The development of PCR methods for the molecular detection of parapox DNA has met the demands for specific and sensitive laboratory diagnosis (Mazur et al., 2000; Guo et al., 2004; Tryland et al., 2005).

Fig. 1. Camel calf of six months of age exhibiting typical symptoms of contagious ecthyma.

Confirmation of recombinants was done by restriction analysis and positive clones were sequenced. The nucleotide sequences of the topoisomerase gene were submitted to GenBank, NCBI database, for which the assigned accession No. is HQ844268. The open reading frame (ORF) of PCPV camel is 957 bp in length encoding 318 amino acid polypeptide. The ORF has two asn-glycosylation motifs at position 229 and 311 as is the case for the remaining seven parapoxviruses analyzed in the study. Similarly, myristylation (at position 227) motifs and amidation (at postion 65) motifs were also conserved among all the eight parapoxviruses analyzed. Overall, there is no variation within the functional motifs of the topoisomerase gene of all the eight parapoxviruses studied.

A phylogenetic tree constructed using nucleotide sequences of the topoisomerase gene of various parapoxviruses revealed that the Indian PCPV clustered with other parapoxviruses published earlier, supported by high bootstrap values (Fig. 2). The gene had a high G + C content (63.06%), consistent with the relatively high G + C content in the whole genome of parapoxviruses (Delhon et al., 2004). We compared the sequence of Indian PCPV camel with all seven sequences representing ORFV, PCPV and BPSV available in the database. Sequence analysis revealed that Indian PCPV shared 95.2–95.5% and 95.9–96.8% sequence identity at the nucleotide and amino acid level, respectively, with PCPV reindeer. ORFV from different regions of the world shared 92.3–92.7% and 96.2–96.5% sequence identity at the nucleotide and amino acid level, respec-