Haryana Vet. **47** (December, 2008), pp 41-45

Research Article

VP7 GENE BASED MOLECULAR CHARACTERIZATION OF TWO INDIAN ISOLATES OF BLUETONGUE VIRUS

SANGEETA DALAL[1], G. PRASAD, MINAKSHI and SUSHILA MAAN
Department of Animal Biotechnology, College of Veterinary Sciences
CCS Haryana Agricultural University, Hisar -125 004

ABSTRACT

In the present study, vp7 gene specific primer pair was used to amplify two cell culture adapted Indian isolates of bluetongue virus (BTV-1 Avikanagar, BTV-15 Hyderabad). The purified and polished 1137 bp and 1154 bp PCR products of BTV-1 (Avikanagar) and BTV-15 (Hyderabad) were cloned into PCR-Script Amp SK (+) vector. The recombinant clones were screened for the presence of insert using restriction endonuclease digestion (*Xho1*) and PCR assays with vp7 gene specific primers. The nucleotide sequence analysis revealed that the two Indian BTV isolates showed a 79 - 99.9% nucleotide identity. Furthermore, the phylogenetic analysis of Indian isolates tree showed that the BTV-1 Avikanagar formed a monophyletic group with BTV-18 and BTV-23 Indian isolates and cluster together with South African, Australian and Chinese isolates of serotype 1, 2, 4 while BTV-15 (Hyderabad) falls in a separate cluster along with BTV-10 and 17 from US. This study suggested that the vp7 gene based phylogenetic tree depicts grouping of Indian BTV isolates at random and this gene does not assort according to topotype or serotype, even over large geographic distances.

Key words: Bluetongue virus, orbivirus, reverse transcription polymerase chain reaction, topotype, VP7 gene

Bluetongue virus (BTV) causes an economically important disease in domestic and wild ruminants. In addition, bluetongue (BT) is an OIE notifiable disease, hence there are mandatory restrictions on the movement of livestock and their products between BT endemic and BT free countries (Osburn, 1994). The BTV, member of genus *Orbivirus*, family *Reoviridae*, is an icosahedral shaped, non-enveloped particle consisting of 10 segmented ds RNA genome (Verwoerd, 1969), encapsidated in a double layered protein coat. The inner capsid or core is made up of two major structural proteins VP3 and VP7 and three minor structural proteins VP1, VP4 and VP6 whereas the outer shell or the outer capsid consists of VP2 and VP5 proteins (Verwoerd *et al.*, 1972). Non-structural proteins viz. NS1, NS2, NS3 and NS3a of BTV genome are produced in BTV infected host cells only. The RNA genome segment 7 of BTV encodes a moderately conserved, major group specific immuno-dominant protein VP7 showing serological cross reactions between different isolates within individual Orbivirus serogroups (Huismans and Erasmus, 1981, Gumm and Newman, 1982). Changes in VP7 protein may be observed due to its requirement to accommodate the more variable outer capsid proteins. Therefore variation observed in segment 7 / VP7 could also result from a certain level of antibody selection pressure. The serogroup specific genes of BTV such as VP3 (Gould and Pritchard, 1990), VP7 (Bonneau *et al.*, 2000, Wilson *et al.*, 2000 and S10 (Pierce *et al.*, 1998, Bonneau *et al.*, 1999, Nikolakaki *et al.*, 2004) are the good targets for topotyping or geotyping. Therefore, BTVs within a region can be differentiated with the help of these serogroup specific genes (Gould *et al.*, 1992). The VP7 protein being responsible for cell tropism of BTV in the insect vector, it would have been evolving with the different *Culicoides* species present in various regions of the world to form distinct topotypes (Bonneau *et al.*, 2000). There are only few reports on genome sequence based molecular characterization of Indian isolates so far (Tiwari *et al.*, 2000, Dahiya *et al.*, 2004, Kovi *et al.*, 2006). Keeping this in mind, the present

[1]Corresponding author, University of Saskatchewan, Saskatoon (SK), Canada S7N5A8
E mail: sad950@mail.usask.ca

work on vp7 gene based molecular characterization and phylogenetic analysis of two Indian isolates, belonging to distinct geographical regions of India was undertaken.

MATERIALS AND METHODS

***Viruses*:** The BTV-1 Avikanagar (BTV-1A, passaged approximately 60 times) was isolated from Central Sheep and Wool Research Institute, Avikanagar, Rajasthan (Prasad *et al.*, 1994) while BTV-15 Hyderabad (BTV-15 Hyd, passaged approximately 15 times) was isolated from Iskilla village, Nalgonda, Andra Pradesh. Both Indian isolates were propagated separately into a day old BHK-21 cell line grown using MEM medium supplemented with L-glutamine, newborn calf serum and antibiotics. When the infected BHK-21 monolayer showed about 75 - 90 % cytopathic effect (CPE), infected BHK-21 cell suspension (without freezing and thawing) was centrifuged to obtain a cell pellet containing cell associated BTV.

***Extraction of viral RNA*:** The viral genomic RNA of BTV propagated in BHK-21 cell line was extracted using Trizol method (Sambrook and Russell 2001a).

***RT-PCR using vp7 gene terminal sequences specific primers*:** The 25 µl reaction mixture was standardized with cell culture grown two Indian isolates of BTV, according to method described by Kovi *et al.* (2006).

***Polymerase chain reaction*:** The 1.25 U Platinum Taq DNA polymerase (Invitrogen) was used for the PCR generating full-length cDNA using vp7 gene specific terminal primers. The 25µl PCR reaction mixture generated contains 5µl of cDNA along with 5 % DMSO, 25 pmol of each primer, 200 mM of dNTPs, and 1.25 µM of $MgCl_2$. The optimum conditions to get specific product of 1154 bp (BTV-15 Hyd) and 1137 bp (BTV-1 A) were observed when the PCR reaction was carried out with initial denaturation at 95° C for 2 min and 30 cycles each having denaturation at 94° C for 45 sec, annealing at 56° C for 1 min, extension at 72° C for 1.30 min and final extension at 72° C for 10 min. The expected PCR products were then visualized by electrophoresis in agarose gel electrophoresis with ethidium bromide.

***Cloning of vp7 gene of BTV*:** The cloning strategy was followed as per the manufacturer's instructions for PCR-Script TM Amp cloning kit from Stratagene. Briefly the purified and polished PCR product of BTV isolates, BTV-1A (1137 bp) and BTV-15 Hyd (1154 bp), was digested with restriction enzyme and ligated before transformation into XL10-Gold® Kan ultracompetant *E. coli* cells.

***Analysis of transformants*:** Blue-white colour screening along with the colony touch PCR with suitable primers using the same cycling temperature profile as for the BTV vp7 gene amplification was used for identification of recombinant clones. After analyzing the PCR product in AGE, positive clones were selected for further studies.

***Isolation of plasmid DNA and the in-vitro restriction endonuclease profile analysis*:** Minipreparation of plasmid DNA from recombinant bacteria was done by alkaline lysis method described by Sambrook and Russell (2001 b) with minor modifications. The recombinant clones of vp7 gene of the two Indian isolates of BTV were subjected to restriction digestion with *Xho1* (MBI Fermentas) as per the manufacturer's instructions.

***Nucleotide sequence and Phylogenetic analysis*:** The BTV-1A (1137 bp) and BTV- 15 Hyd (1154 bp) were subjected to sequencing using automated DNA sequencing facility (ABI PRISM TM version 3.0, Model 3100) at Department of Biochemistry, University of Delhi, South Campus, New Delhi. The Universal primers, T7 and T3 were used for initial sequencing of BTV-1A and BTV-15 Hyd respectively. Also subsequently internal/ walking gene specific primers were designed for BTV-1A and BTV-15 Hyd to complete the internal approximately 100 nucleotides of the vp7 gene sequence of both the isolates. All the sequence analyses were done after converting each sequence into FASTA format as available on Internet (http:// www.ncbi.nlm.nih.gov). The vp7 gene sequences of Indian isolates used in this study were subjected to BLAST search (http:// www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html). The sequence data obtained were used to

generate a contiguous sequence and the final contiguous sequences of vp7 gene of Indian isolates as well as the other published vp7 gene sequences of BTV and related orbiviruses were then aligned using computer soft wares, Clustal W and Clustal X ver. 1.81 (Thompson *et al.*, 1994, 1997). Uncorrected ("p") distance matrix was also calculated for both BTV isolates described above using Clustal X ver. 1.81 programme. The multiple sequence alignment and phylogenetic analysis (construction of neighbor-joining, NJ) was performed using software Clustal X (ftp://ftp-igbmc.u-strasbg.fr/pub/Clustal X) and Treeview (win 32) (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

RESULTS AND DISCUSSION

The present study of vp7 gene based molecular characterization of two Indian isolates of BTV was conducted by generation of recombinant clones of vp7 gene in *E. coli* and their phylogenetic analysis. The amplification obtained was specific to vp7 gene and no spurious products were observed (Fig 1). Cloning of vp7 gene of BTV-1 A and BTV-15 Hyd in PCR-Script Amp SK (+) vector system (Stratagene) yielded majority of the white colonies (with insert) and generated an expected sized amplicons of 1154 bp for BTV-15 Hyd and 1137 bp for BTV-1A after touch PCR in 1% gel. However, the blue colonies, some of white colonies and untransformed competent cells did not yield any

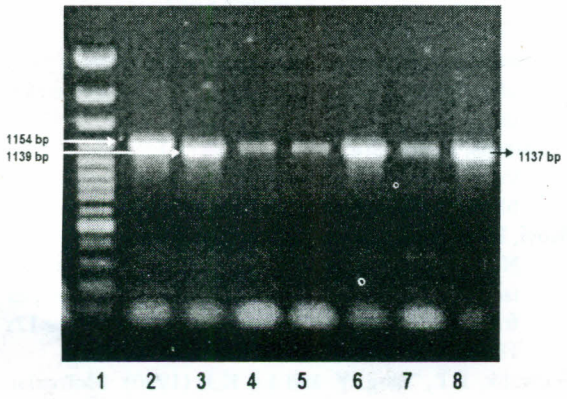

Fig 1. Amplifcation of full-length VP7 gene of Indian BTV isolates by RT-PCR.
Lane 1: 100 bp DNA ladder Lane 2: BTV- 15 Hyderabad
Lane 3: BTV- UT (Kolkata) Lane 4: BTV-1Sirsa 1
Lane 5: BTV- 1Sirsa 3 Lane 6: BTV-1 Avikanagar
Lane 7: BTV- 23 IVRIa Lane 8: BTV-23 IVRI3

amplification. The plasmid isolation of white colonies carried out by minipreparation, yielded plasmid of two sizes ranging between 2.5 kb and 5.0 kb i.e. two forms of plasmid viz., supercoiled, nicked/open circular which migrated at different speeds in 1% gel according to their molecular weights and conformation. The blue colonies yielded two bands of approx. 1.8 kb and 3.5 kb. The plasmids isolated from blue and white colonies yielded single band of 2961 bp and 4098 bp, respectively after digestion of PCR-Script Amp SK (+) vector with *Xho1* as it has a single target site at sequence CTCGA↓G in pBluescript SK+ vector and no site in the vp7 gene inserts (Fig 2). The nucleotide sequence data analyses

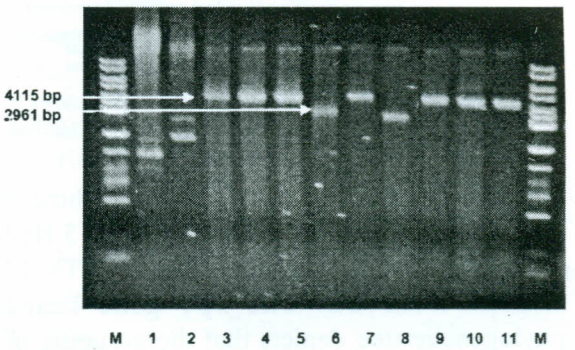

Fig 2. Restriction digestion of recombinant plasmids with *XhoI*. Lane M: 1 kb DNA ladder, Lane 1: Uncut blue colony, Lane 2: Uncut white colony, Lane 3: BTV-15 Hyderabad, Lane 4: BTV-UT (Kolkata), Lane 5: BTV-1-Sirsa1, Lane 6: Cut blue colony, Lane 7: BTV-1-Sirsa3, Lane 8: Cut blue colony; Lane 9: BTV-1-Avikanagar, Lane 10: BTV-23 IVRI 1, Lane 11: BTV-23 IVRI 3

have shown that although the BVT-1A and BTV-15 Hyd vp7 genes have 21% nucleotide sequence divergence yet they have same length of genome segment 7 (total length 1154 bp). However, in this study the vp7 gene length was 1137 nucleotides as the amplification of this genome segment was carried out using 5' forward primer from coding sequence (i.e P3 designed from 18th nucleotide onwards). All the earlier Indian isolates have very low sequence similarity with American, Chinese and French isolates but they have high sequence similarity with Australian and South African isolates (Kovi *et al.*, 2006). The tree generated after phylogenetic comparison showed that the BTV-1A formed a monophyletic group with BTV-18 and BTV-23 Indian isolates (Fig 3). The BTV-1A isolates cluster together

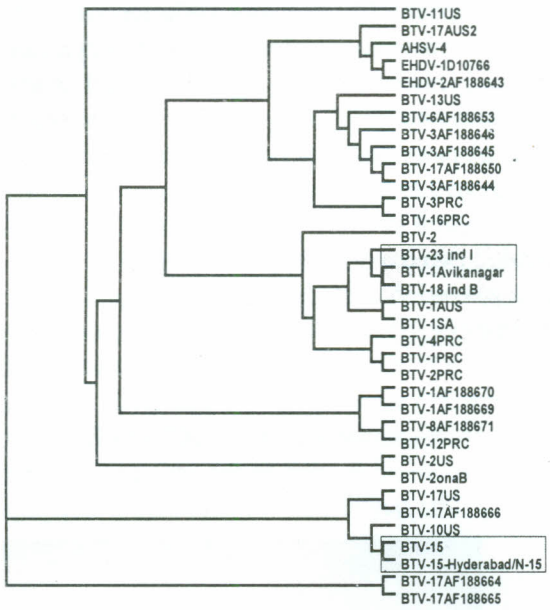

Fig 3. The nucleotide sequence alignment of VP7 gene of BTV-1 Avikanagar and BTV-15 Hyderabad (Indian isolates of BTV)

with South African, Australian and Chinese isolates of serotype 1, 2, 4 while BTV-15 Hyd falls in a separate cluster along with BTV-10 and 17 from US. The vp7 gene based phylogenetic tree depicts that the grouping of BTV isolates is at random and this gene does not assort according to topotype or serotype, even over large geographic distances (Fig 3). Hence, though the vp7 gene is highly conserved, apparently it was not useful target for topotyping. Kowalik *et al.* (1990) cloned and sequenced vp7 gene of all the five US serotypes of BTV 2, 10, 11, 13 and17, and reported that there was 7-20% nucleotide sequence divergence among them with BTV-13 being the most divergent serotype. In yet another previous study, Wilson *et al.* (2000) have reported that although the BTV isolates studied showed a 96.7-99.2% amino acid identity, they segregated into five clades and did not display an unequivocal geographic, temporal or serotype relationship. Another phylogenetic analysis of the vp7 gene of BTV isolates (Bonneau *et al.*, 2000) from China, Australia, South Africa and U.S. also did not show a geographic or serotype relationship despite the potentially critical role of VP7 protein in virus-vector interactions.

From this study it can be concluded that the two Indian isolates of different serotypes belonging to two different regions fall in different clusters. In addition, the sequence comparison studies also revealed that Indian isolates might have originated from South African or Australian strains. Hence, it can be understood that other conserved gene of BTV might be more useful in topotying than the vp7 gene. As all genome segments of BTV can not be used for topotyping, it suggests that these genes evolve independently of one another, in response to the selective pressures encountered with in their respective insect and vertebrate hosts.

REFERENCES

Bonneau, K.R., Zhang, N.Z., Wilson, W.C., Zhu, J.B., Zhang, F.Q., Li, Z.H., Zhang, K.L., Xiao, L., Xiang, W.B and MacLachlan, N.J. (2000). Phylogenetic analysis of the S7 gene does not segregate Chinese strains of bluetongue virus into a single topotype. *Arch. Virol.* **145**: 1163-1171.

Bonneau, K.R., Zhang, N., Zhu, J., Zhang, F., Li, Z., Zhang, K., Xiao, L., Xiang, W. and MacLachlan, N.J. (1999). Sequence comparison of the L2 and S10 genes of bluetongue viruses from the United States and the Peoples Republic of China. *Virus Res.* **61**: 153-160.

Dahiya, S., Prasad, G., Minakshi, and Kovi, R.C. (2004). Vp2 gene based phylogenetic relationship of Indian isolates of bluetongue virus serotype different parts of the world. *DNA Sequence* **15**: 351-361.

Gould, A.R., McColl, K.A. and Pritchard, L.I. (1992). Phylogenetic relationships between bluetongue viruses and other orbiviruses. In: African Horse Sickness and other Related Orbiviruses. CRC Press, Boca Raton. pp. 452-460.

Gould, A.R. and Pritchard, L.I. (1990). Relationships amongst bluetongue viruses revealed by comparisons of capsid and outer coat protein nucleotide sequences. *Virus Res.* **17**: 31-52.

Gumm, I.D. and Newman, J.F.E. (1982). The preparation of purified bluetongue virus group specific antigen for use as a diagnostic reagent. *Arch. Virol.* **72**: 83-93.

Huismans, H. and Erasmus, B.J. (1981). Identification of the serotype specific and group specific antigen of bluetongue virus. *Ondersteport J. Vet. Res.* **48**: 51-58.

Kovi, R.C., Dahiya, S., Prasad, G. and Minakshi (2006). Nucleotide sequence analysis of VP7 gene of indian isolates of bluetongue virus vis-a-vis other serotypes from different parts of the world. *DNA Sequence* **17**: 187-198.

Kowalik, F.T., Yang, Y. and Li, K.J. (1990). Molecular cloning and comparative sequence analyses of bluetongue virus S1 segments by selective synthesis of specific full-length DNA of ds RNA genes. *Virol.* **177**: 820-823.

Nikolakaki, S.V., Nomikou, K., Mangana-Vougiouka, O., Papanastassopoulou, M., Koumbati, M. and

Papadopoulos, O. (2004). S10 segment sequence analysis of some Greek BTV strains. In: Bluetongue Part II Proc. III International Symposium. Taormina 26-29 October 2003. *Veterinaria Italiana* **40**: 468-472.

Osburn, B.I. (1994). The impact of bluetongue virus on reproduction. *Comp. Immunol. Microbiol. Infect. Dis.* **17**: 189-196.

Pierce, C.M., Balasuriya, U.B. and MacLachlan, N.J. (1998). Phylogenetic analysis of the S10 gene of field and laboratory strains of bluetongue virus from the United States. *Virus Res.* **55**: 15-27.

Prasad, G, Garg, A.K., Minakshi, Kakker, N.K. and Srivastava, R.N. (1994). Isolation of bluetongue virus from sheep in Rajasthan. *Rev. Sci. Technol. Office of Int. Epizootics* **13**: 935-937.

Sambrook, J. and Russell, D.W. (2001a). Extraction purification and analysis of mRNA from eukaryotic cells. In: Molecular Cloning: A Laboratory Manual. (3rd edn.), Cold Spring Harbor Laboratory Press, New York. pp. 7.1-7.94 A8.19-A8.21.

Sambrook, J. and Russell, D.W. (2001b). Plasmids and their usefulness in Molecular Cloning. In: Molecular Cloning: A Laboratory Manual. (3rd edn.), Cold Spring Harbor Laboratory Press, New York. pp. 1.1-1.34.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**: 4876-4882.

Thompson, J.D., Higgins, D.G. and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673-4680.

Tiwari, A.K. Kataria, R.S., Desai, G., Butchaiah, G. and Bandyopadhyay, S.K. (2000). Characterization of an Indian bluetongue virus isolate by RT-PCR and restriction enzyme analysis of the VP7 gene sequence. *Vet. Res. Commun.* **24**: 401-409.

Verwoerd, D.W., Els, H.J., Devillers, E.M. and Huismans, H. (1972). Structure of the bluetongue virus capsid. *J. Virol.* **10**: 783-794.

Verwoerd, D.W. (1969). Purification and characterization of bluetongue virus. *Virol.* **38**: 203-212.

Wilson, W.C. Ma, H.C., Venter, E.H., Van Djik, A.A., Seal, B.S. and Mecham, J.O. (2000). Phylogenetic relationships of bluetongue viruses based on gene S7. *Virus Res.* **67**: 141-151.