

VP7 GENE BASED PHYLOGENETIC ANALYSIS OF BLUE TONGUE VIRUS SEROTYPE 15

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Abstract: The present study was under taken with a view to characterize BTV 15(N12) isolate employed for trial vaccine. BTV 15 virus was cultivated in BHK₂₁ cell line and RNA was isolated. Further, RT-PCR was standardized for detection of VP7 gene of BTV 15. Molecular characterization of the isolate was taken up by the sequencing of VP7 (segment 7). The sequences were compared to the available sequences in Genbank. Further analysis indicated that all VP7 nucleotide sequences segregated into 7 phylogenetic clades. Results indicated that BTV-15 (N12) was more closely related to BTV-15 (N15), BTV-15 (DQ399835), BTV-15 China than to BTV 15 Australia and BTV-9 (MBN isolate). From this study it can be concluded that the BTV-15 Indian isolate might have been originated from Chinese strains.

Keywords: Bluetongue virus, VP7 gene and Molecular characterization.

Introduction

Bluetongue is an economically important disease of domestic and wild ruminants. The disease is caused by bluetongue virus belonging to the genus Orbivirus of family Reoviridae (Sairaju V *et al.*, 2013). The disease is transmitted by culicoides midges and is endemic where culicoides species can survive (Susmitha *et al.*, 2012). Twenty seven serotypes of BTV are recognized worldwide (Jenckel M *et al.*, 2015, Maan NS *et al.*, 2012., Rao PP *et al* 2012). The BTV genome is composed of ten linear segments of double-stranded RNA (dsRNA). The 10 segments code for seven structural (VP1-VP7) and four non structural proteins (NS1,2,3,3A) (Bommineni *et al.*, 2008., Rao PP *et al* 2012., Maan NS *et al.*, 2012.). The VP7 protein is not only a major group reactive antigen, but also a good stimulator of B cells mediated immune response (Jeggo and Wardy 1986). In BTV infected animals majority of the antibody response is against the group specific antigen VP7 (Joseph and Yang 1990). The VP7 protein being responsible for cell tropism of BTV in the insect vector, would have been

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evolving with the different culicoides species present in various regions in the world to form distinct topotypes (Bonneau *et al.*, 2000). Detailed information on this gene may be useful for characterizing the strain, genotyping, vaccine production and development of drugs. In the present study, VP7 gene based phylogenetic analysis of Bluetongue virus serotype 15 was studied.

Materials and methods

The BTV serotype 15 serotyped at serotyping Centre, All India Net Work Project on Bluetongue disease, Haryana Agricultural University, Hissar was used in this study. Bluetongue virus was passaged five times in BHK21 cell lines. The RNA was isolated from BTV infected cell cultures by Acid Phenol method (Chomczynski and Sacchi (1987)). The quality of isolated RNA was checked by agarose gel (1% w/v) electrophoresis. VP 7 gene was amplified by using the set of primers described by Wade Evans *et al.* (1990) based on segment 7 sequence of BTV-1 South Africa (GenBank Acc. No. X53740). The primers were S7(1-17)- F 5'GTAAAAATCTATAGAG3' and S7(1156-1138) -R 5'GTAAGTGTAATCTAAGAGA3'. The product size was 1156 bp. For cDNA synthesis RNA mix was prepared by adding 1µl primer A(20 pmol/µl) and 1µl Primer B (20 pmol/µl) to 8 µl of isolated RNA. The mix was heated at 68⁰C for 5 min. and snap cooled on ice. cDNA mix was prepared by adding 4.0µl of 5XRT buffer, 1 µl of DTT(0.1M), 0.5 µl of RNase out(40U/ µl), 1.0 µl of AMV RT(15U/ µl), 2.0µl of dNTPs and 1.5 µl of ultra pure water. cDNA mix was added to RNA mix, incubate at 25⁰c for 15min and 42⁰C for 45min then kept at 4⁰c. cDNA of VP7 genes was amplified by Polymerase chain reaction as per the protocol of Wade Evans *et al.* 1990 with some modifications. The PCR mix consisted 5µl of 10X PCR buffer, 5µl of Magnesium chloride (25mM), 1µl of 10mM dNTPs, 1µl of taq DNA polymerase (5U/µl), 31 µl of Nuclease free water, 5µl of cDNA and 1µl(20pM) each of forward and reverse primers. The PCR was carried out by thermal cycler machine (Thermo scientific). The cycling conditions were initial denaturation at 95⁰C for 3min, 30 cycles of 95⁰C/20sec, 39⁰C/60sec primer annealing and 70⁰C/2min extension followed by final extension of 70⁰C for 7min. After completion of the cycles the PCR product obtained was subjected to 1% agarose gel electrophoresis. PCR product was purified with QIA quick gel extraction kit (cat no. 28704) as per the manufacturer's instructions with suitable modifications. Purified PCR product was sequenced at M/s Bioserve Biotechnologies Ltd, Hyderabad, using PCR sequencing method in Beckman CEQTM 8000 Genetic Analysis System using S7 gene specific primers. The sequence of segment 7 of BTV 15 isolates

obtained after sequencing were then aligned with database sequences using NCBI BLAST (www.ncbi.nlm.nih.gov/blast) to confirm their identity. Further, nucleotide sequence was multiple aligned using CLUSTAL W (Thompson *et al.*, 1997) with 75 different BTV VP7 gene sequences, obtained from GenBank. All available sequences were loaded in CLUSTAL W (www.ebi.ac.uk/clustalw) software to obtain the cladogram.

Results and discussion

Double stranded RNA isolated from BHK-21 cell cultures infected with BTV-15 isolate was analyzed by agarose gel electrophoresis and revealed segmented genome pattern with 10 bands including two inseparable bands (Fig 1). Segmented pattern of genome is characteristic feature of BTV nucleic acid (Verwoerd *et al.*, 1972; Squire *et al.*, 1983 and Roy 1992). Resolving RNA bands were not clear for two segments. RT-PCR was standardized for VP7 gene of BTV serotype 15 (N12). A specific band of 1156bp was observed (Fig 2) as expected. The sequence of the isolate was compared with available sequences in the database (genbank) using clustal W software.

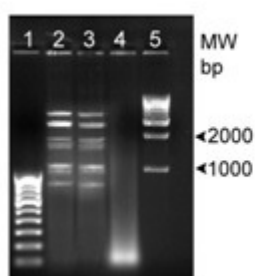


Fig:1 Migration patterns of the BTV – 15 (N12)

Lane:1 100bp ladder
Lane:2 and 3: BTV – 15 (N12) ds RNA
Lane: 4 BHK21 Cell RNA
Lane 5: 1kb ladder

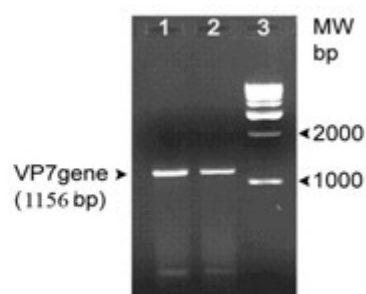


Fig: 2 VP7 gene PCR product of BTV -15 (N12)

Lane:1 & 2 BTV-15 (N12)
Lane :3 1 kb ladder

The percentage of identity of isolate with Australian BTV-15 strain, Chinese BTV-15 strain and BTV strain of Taiwan was 76, 63 and 76%. Phylogenetic analysis revealed that VP7 gene sequence of available BTV serotypes can be distributed into 7 clades (Fig 3). The isolates of BTV-2 and BTV-15 are segregated into clade 1 (Fig 3). Further analysis of segment 7 revealed that there was 73% homology between BTV-15 (N12) and BTV-2 (M11). BTV-15 (N12) was closely related to BTV-15 (N15), BTV-15 (DQ399835), BTV-15 China than to BTV-15 Australia and BTV-9 (MBN). Nucleotide homology of S7 segment between Indian isolates of BTV-2, 9 and BTV-10 US was 79% (Prasad 2005). Dalal *et al.* (2008) observed that BTV-15 was closely related to BTV-10 and 17 from USA. Kowalik *et al.* (1990) cloned

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