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Purification and characterization of BTV-VP7UD protein

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ABSTRACT
Bluetongue is a non-contagious arthropod born viral disease of small ruminants. The diagnosis of the infection is carried by serological methods, which in turn may produce cross reaction. In order to prevent this monoclonal antibodies has risen which were cost effective, the viral protein like particles are used to diagnosis as well as sub unit vaccine to prevent the infection. The BTV-VP7 is an important structural protein conserved in most of the serotypes known so far. The truncated upper domain portion of BTV-VP7 is further much conserved, so the present study is carried to develop a diagnostic kit by e producing viral protein of BTV-VP7Upper domain.
***Key words**: BTV,VP7, Upper domain, expression, purification and chloroform.*

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INTRODUCTION

Bluetongue virus (BTV), is an arthropod vector borne non contagious of small ruminants. The genome is made up of 10 linear segmented ds-RNA, which codes for seven structural and five nonstructural proteins [1]. The VP7 protein is a structural protein, which is a group specific antigen and conserved in all serotypes [10]. The VP7 is made up of two portion upper and lower domain, the upper domain is have amino terminal half of the VP7 is conformational and accessible on the virus surface [6-8].The present study was designed to amplify the group specific antigen i.e., VP7 upper domain gene of BTV and it was expressed in the prokaryotic system in order to develop the detection kit.

MATERIAL AND METHODS

Amplification and cloning of VP7UD:

Amplification of the BTV-VP7 gene upper domain of BTV-9 isolated by Bommineni *et al.*,[3]. This was propagated in BHK-21 cell lines, after development of proper CPE, RNA isolation was carried out [4]. This RNA was used for the amplification of cDNA of VP7UD with primers having restriction digestion enzymes HindIII and ECoRI using annealing temperature at 55°C for 30sec. This amplified gene of VP7UD was cloned in to pQE-30 vector for multiplication. Both pQE-30 vector and VP7UD gene treated with the retraction digestion enzymes HindIII and ECoRI. These were subjected for ligation at 16^0C by using T4 DNA ligase enzyme for overnight. These ligated products transformed into chemically competent *E.coli* DH5α cells [16] and were propagated by using LB agar plates with Ampicillin (100µg/ml) and Kanamycin (50µg/ml).

The colonies were analyzed by using 1% agarose the transformed colonies with ligated product were ran slowly than the non-transformed cells. These were propagated in LB broth with Ampicillin (100µg/ml) and Kanamycin (50µg/ml) and the genome was extracted [16] and confirmed by using RE digestion with release of pQE-30 as well as VP7UD by using 1% agarose gel electrophoresis.

Expression of VP7UD:

This was transformed into *E.coli* M15 cells for the expression of VP7UD; the transformed product was subjected for expression by using 1mM IPTG [11]. The expression of these induced cells was analyzed by using 10% SDS-PAGE.

Purification of expressed protein:

The purification of the expressed protein was carried according the protocol given by Sambrook and Russel [16], the purification was analyzed by 10% SDS-PAGE, which shows some unwanted protein of *E.coli* M15 cell wall protein. In order to purify this it was subjected to wash with chloroform @ 10% volume of the cell lysate, kept at 4^0C for overnight. This chloroform treatment was carried for two times and finally washed with wash buffer results in the obtaining of purified product upon analysis of 12% SDS-PAGE. 200ng of this product was used for raising hyper immune serum in rabbit.

RESULTS

Amplification of the BTV-VP7 gene upper domain This was propagated in BHK-21 cell lines, after RNA was used for the amplification of cDNA of VP7UD with primers having restriction digestion enzymes HindIII and ECoRI using annealing temperature at 55°C for 30sec was analyzed by 1% agarosegel with M.Wt 387bp (Fig:1).

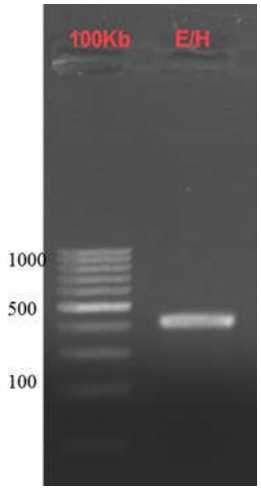

Fig:1-LANE 1 : DNA LADDER 100bp, LANE 2: PCR PRODUCT (387bp)

This was cloned in to pQE-30 vector, The cloned colonies were analyzed by using 1% agarose the transformed colonies with ligated product were run slowly than the non-transformed cells (Fig: 2).

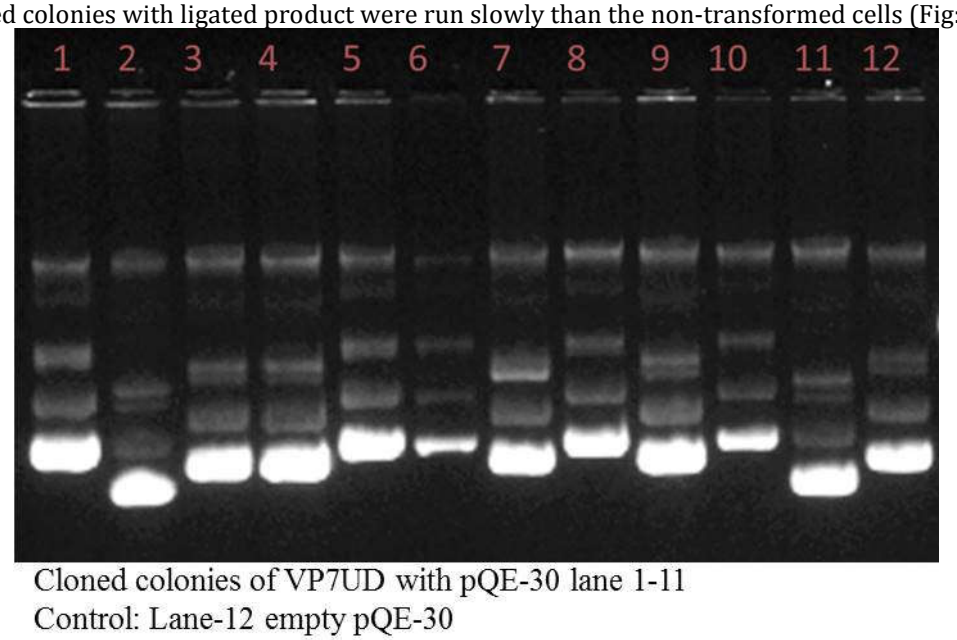

Fig 2: Lane 1, 5, 6 and 10 ran slowly with compared to empty vector in lane-12. These were propagated in LB broth with Ampicillin (100µg/ml) and Kanamycin (50µg/ml) and the genome was extracted and confirmed by using RE digestion with release of pQE-30 as well as VP7UD by using 1% agarose gel electrophoresis.

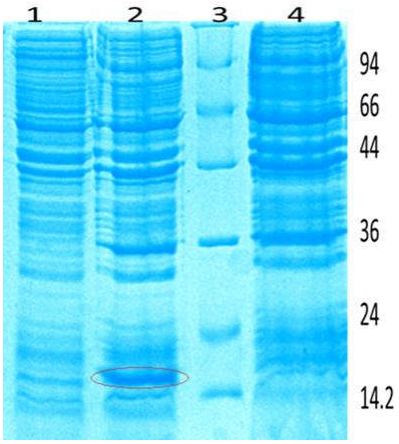

Fig:3: LANE 1 UNINDUCED VP7 UPPER DOMAIN , LANE 2 INDUCED VP7 UPPER DOMAIN LANE 3 MARKER (94-14.2 K Da) LANE 4 EMPTY VECTOR pET 15 *E.coli* cells

The purification was carried by protocol of insoluble proteins purification and the purification was analyzed by 10% SDS-PAGE, which shows some unwanted protein of *E.coli* M15 cell wall protein (Fig:4) with M.Wt of 14.5KDa.

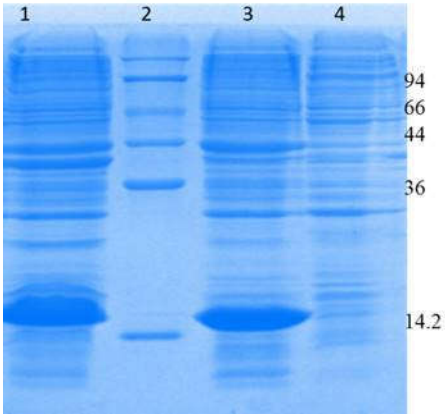

Fig:4: LANE 1 and 3: VP7 UPPER DOAMIN PROTEIN; LANE 2: MARKER (14.2- 94 KDa) LANE 4: WASHBUFFER SUPERNATANT

The purified product upon treat with chloroform gives the purified product without any unwanted cell wall protein (Fig:5) showing band at 14.5KDa.

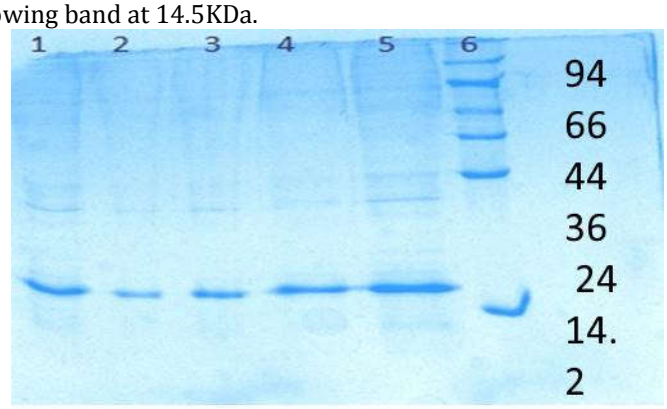

Fig:5: LANE 1-5 PURIFIED VP7 UPPER DOMAIN PROTEIN , LANE 6 MARKER (94-14.2KDa)

Hyper immune serum was developed by using this purified product in rabbit

DISCUSSION

BTV is a non-contagious economically important disease of small ruminants, spread by *culicoidessps* [14].Detection of BTV is carried by serological methods which results in giving false results due to cross reactivity. To reduce this cross reactivity monoclonal antibody technique was developed which is a cost effective one. For this there is need to development of recombinant protein which were used both as diagnostic as well sub unit vaccine to prevent infection [13]. The group specific antigen of BTV-VP7, which is conserved in all serotypes, was target for the present study as VP7 contains the neutralizing antibodies and is conserved in all the known serotypes. Prokaryotic expression was carried by using the *E.coli* M15 cells. Group-specific antigen expressed by various laboratories in baculovirus, E. coli, yeast or vaccine virus [5, 12, 13, 15], has been widely employed in BT diagnostics. Baculo virus expressed triple and quadruple recombinant protein and prokaryotic expression of BTV viral proteins [2] are used as diagnostic kits. Recombinant protein VP5 and VP5 in prokaryotic system used to develop subunit vaccine [9].

BTV- 9 was used in the present study isolated by Bommineni *et al* [3], the CPE was observed after 2 days in BHK-21 cell line. The trizol method of RNA isolation carried and amplification of cDNA by RT-PCR with annealing temperature at 55°C for 30sec; analyzed by agarosegel electrophoresis with 387bp. The restriction enzymes ECoRI and Hind III were used for the cloning into pQE-30 vector for amplification of BTV-VP7UD, which on further analyzed by 1% agarose gel electrophoresis; the clonrd colonies were identified by slow running colonies when comparing the uninoculated genome of *E.coli* DH5α cells. These were further transformed into *E.coli* M15 cells for expression; the expression was carried by using 1mM IPTG for 6hrs at 37^0C till the OD value reaches 0.6 at200nm. This was purified by using protocol given by Sambrrok and Russel [16] for purification of insoluble proteins as BTV-VP7 is an hydrophobic protein.

The purification of expressed protein containing unwanted cell wall proteins of with treatment of chloroform as it is a lipolyzer. Upon treatment with chloroform @ 10% volume obtained the purified product of VP7UD. The hyper immune serum was raised by using denaturing the protein with 6M Guanidine HCland hyper immune serum was obtained, which western blottingfailed to detect the native virus. Further studies were going on to develop the diagnostics with VP7UD.

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