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CLONING AND EXPRESSION OF BLUETONGUE VIRUS VP7 PROTEIN UPPER DOMAIN IN PROKARYOTIC SYSTEM

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Abstract

Bluetongue (BT) is an infectious non-contagious viral disease of ruminants mainly confined to sheep, caused by Blue tongue virus belongs to *Reoviridae*. It comprises of 10 segmented ds-RNA, each encodes for specific structural or non structural proteins. Among the structural proteins VP7 is an important protein which is a group specific antigen and highly conserved in all the known serotypes. Keeping this in consideration present study was conducted on expression of upper domain of VP7 of BTV. This BTV VP7 UD gene was amplified by RT-PCR and was cloned and expressed with pQE-30 vector in *E.coli* M15 cells. The protein was expressed by 1mM IPTG and was estimated by 12%SDS-PAGE with expected molecular weight at 14.9K.Da. The recombinant VP7UD was further analysed by using western blot, which failed to detect naïve blue tongue virus.
Key words: BTV-9, VP7 upper domain, Expression, *E.coli* M15 cells

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Introduction

Bluetongue (BT) is a non-contagious, arthropod-borne viral infectious disease of ruminants caused by bluetongue virus (BTV), of the genus and the family *Reoviridae*. The bluetongue virus genome is composed of 10 linear segments of double stranded RNA, which encode 7 viral proteins [VP1-VP7] and five non-structural proteins [NS1, NS2, NS3 and NS3A, NS4] (Huismans and van Dijk 1990, Belhouchet *et al.*,2011). The VP7 protein of BTV is a group-specific antigen and highly conserved across all serotypes (Kowalik and Li, 1991). The upper domain contains epitopes at the amino terminal half of the VP7 is conformational and accessible on the virus surface (Eaton et al. 1991).

Materials and methods

BTV-9 serotype isolated by Bommineni *et al.*, (2008) and maintained in department of Microbiology, CVSc Rajendranagar was used for present work. This BTV-9 sero type was propagated in BHK-21.

Amplification of BTV9 VP7 upper domain gene:

The RNA isolation was carried by using Trizol method (Chomczynski 1971). The RNA was than subjected to reverse transcription for production of cDNA at 25°C for 10minutes followed by 37°C for 60mints by using MMLV-RT enzyme. The cDNA used for VP7UD gene was amplified by using jump start AccuTaq polymerase. The PCR reaction mixer consists of 10 µl of cDNA, 1 µl (20 pmol / µl) each of forward and reverse primers each, 2.5 units of the Jump Start Accutaq polymerase enzyme (sigma code D 5809), 5 µl of the 10X Acctaq PCR buffer, 1µl of the dNTP mix (2mM), and PCR grade water was added to made final volume to 50µl. the PCR conditions were Initial denaturation done at 94°C for 3min.which was followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30sec, extension at 68°C for 1min. and final extension done at 68°C for 10 min.

Primers used

S7UDEcoRIF:
ACACAGATATCATTAAAGAGGAGAAATTAACTATGCCG GCTCGTCAGCCGTAT
S7UDHindR:
TGTACAAGCTTAAAACACAACTTGAATCTGTACCATTGC AT

The amplicon was analysed by using 1%agarose gel.

Cloning of VP7UD gene into pQE-30 vector

The amplified VP7UD gene was cloned into pQE-30 vector for multiplication. Both the VP7UD gene and pQE-30 DNA plasmid were subjected to restriction digestion with EcoRI and HindIII restriction enzymes; and analysed by gel electrophoresis and purification was carried by gel extraction kit. Ligation of these purified products was carried by using T4 DNA ligase enzyme at 16 ºC for overnight. This ligated product was cloned into chemically competent *E.coli* DH5α cells (Sambrook and Russsel 2001). The ligated mixture was added to 100µl of the competent cells incubated over ice for 30mints and heat shock was given at42ºC/90 sec followed by snap cooling on ice. Further 1ml of LB broth was added and incubated for 1hour in a shaking incubator.

Then these cells were plated over LB agar plates containing Ampicillin (100µg/ml) and Kanamycin (50µg/ml) and incubated at 37ºC fro 16h for selection of recombinants.

Confirmation by RE digestion

The transformed colonies were analyzed by restriction digestion with EcoRI and HindIII. The suspected recombinant clones of pQE-30 and VP7UD were inoculated to LB broth containing Ampicillin (100µg/ml) and Kanamycin (50µg/ml) and incubated at 37ºC fro 16h. The cloned plasmids were extracted by phenol chloroform method and subjected to RE digestion with EcoRI and HindIII enzymes. The insert of VP7UD release was checked by using 1%agarose gel.

Expression of pQE-30VP7UD

The recombinant pQE-30VP7UD was transformed in to *E.coli* M15 cells, the positive transformants were selected by RE digestion were inoculated into fresh 100ml LB broth with Ampicillin (100µg/ml) and Kanamycin (50µg/ml) at 1% of inoculum and incubated 200rpm for 16h at 37ºC; until it's OD reaches 0.5 at 600nm.This was expressed after induced with 1mM IPTG (Studier *et al.*, 1990). The expressed cells were pelleted down by centrifugation 7000 rpm for 20min. at 4°C. and were suspended in normal protein washing buffer, expression of these cells were analysed using SDS-PAGE gel Lamelli et al (1970).

Purification of expressed protein:

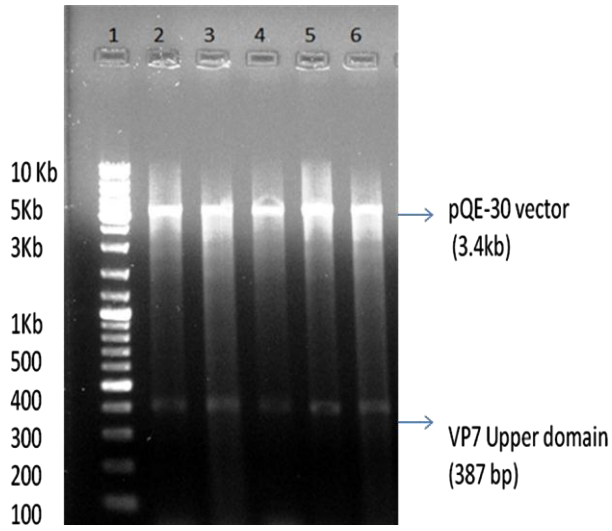
The expressed protein was attempted for purification according to the purification of insoluble proteins as the VP7 is an insoluble protein by using resuspension buffer follwed by wash buffer. (Russel and Sambrook 2001) but this was not purified completely showing other unwanted E.coli proteins, to remove these this washed protein was treated with chloroform. The purified protein thus obtained after 3 washings of chloroform was analysed by using 12% SDS- PAGE electrophoresis.

Rising of hyper immune serum:

The purified protein obtained was denature by using 6M guanidine HCl and dialyzed against 1X PBS. 200ng of this purified protein was injected to rabbits for rising antiserum. This hyper immune serum raised in rabbits was used to detect the BTV naïve virus by western blotting.

RESULTS and DISSCUSSION:

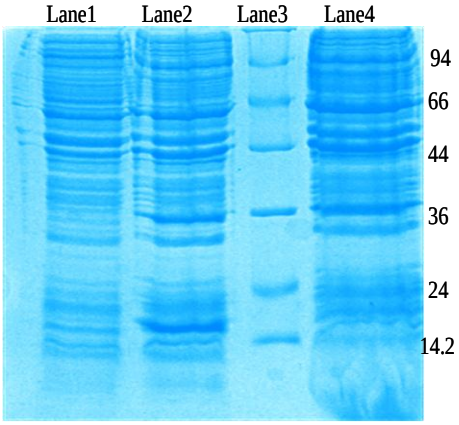
Bluetongue is an economically significant arthropod borne viral disease of domestic and wild ruminants caused by bluetongue virus. The diagnosis of bluetongue virus is mainly relays on the serodiagnosis where they give some cross reactions. The cross reactivity was reduced and overcome by using monoclonal antibodies; but it is very cost effective. To over come there is a need of production of recombinant proteins, which will be used as both diagnostic as well as vaccine (Mertens *et al*., 1984). Hence the present work VP7 structural protein which is highly conserved and group specific one is cloned and expressed in *E.coli* (Yu et al 1988). VP7 is selected because it contains the neutralizing antibodies and is conserved in all the known serotypes, so it is targed for development of vaccine and also the development of diagnostic kits (Mertens *et al*., 1984 and Mertens *et al.*, 1987). The VP7 is highly conserved among all known serotypes among which upper domain portion is preset on the surface of viral particle, Hence the present study was carried on the BTVVP7 upper domain region by using BTV-9 serotype. The BHK-21 cells lines were infected with BTV-9 sero type and was used for the RNA was extraction.. This was subjected to cDNA synthesis in reverse transcription PCR, to amplify VP7UD gene and analysed by using 1% agarose gel showing bands at 387bp and sequencing. The primers were designed along with the suitable restriction enzymes for pQE-30 vector. The upper domain part of the BTV-VP7 was amplified, cloned and expressed in the pQE-30. The cloned colonies grown on LB agar plate containing Ampicillin (100µg/ml) and Kanamycin (50µg/ml) were selected for plasmid isolation and these plasmids were subjected for restriction digestion. The positive samples were confirmed by the release of VP7UD at 387bp (Fig:1).



Lane 1 Marker (1-10kbp)
Lane 2-6: Insert release from cloned plasmids

Fig:1 Confirmation VP7UDpQE-30 with restriction digestion:

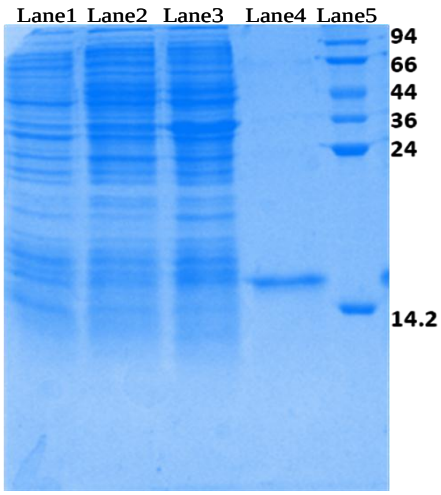
The confirmed plasmids were cloned into *E.coli* M15 cells for expression; with induction of 1mM IPTG for 3h the results were in agreement with (Wang *et al.*, 1996). This expression was analysed by using 12% SDS-PAGE electrophoresis which showing band at 14.5KDa.(Fig:2).



Lane 1 Uninduced Vp7 Upper Domain
Lane 2 Induced Vp7 Upper Domain
Lane 3 Marker (14.2-94 kda)
Lane 4 Empty Vector M15 E.coli cells with pQE-30 vector empty

Fig 2: Expression of recombinant VP7UDpQE-30

This expressed protein was purified by using chloroform (Fig:3). The purified protein thus obtained was used to raise the antibodies in the rabbits and it was identified further by using immunoblotting. This was detecting only the purified recombinant protein but not the naïve BTV-9 virus. Reddy et al. (2006) had also faced such problems and they were unsuccessful to express complete VP7 in bacterial system despite repeated attempts.



Lane 1 to 3 wash supernatants of vp7 u d
Lane 4 purified vp7 ud protein
Lane 5 marker (94-14.2KDa)

Fig 3: Purification of expressed protein:

Wang et al. (1996) cloned full-length VP7 gene of BTV 15 in pET vector and achieved high-level expression in E. coli, but due to the insoluble nature of the expressed protein it was not used for the further processing. Purushotham et al (2013) expressed recombinant proteins of VP5VP7 of bluetongue virus in the prokaryotic system and was recombinant VP7VP5 (117kD) of BTV-16 was expressed in a prokaryotic expression system, BL21 (DE3) pLysS E. coli. The protein was confirmed by Western Blot Analysis. The expressed and purified protein was used to raise the hyper immune serum in rabbits. The hyper immune serum thus raised was analysed by westernblotting with naïve BTV-9 serotype virus, and the purified protein. The hyper immune serum was detect only the purified protein but not identified the virus. The BTV VP7 protein is a highly hydrophobic protein so the purification was very difficult. Even though the BTV VP7 UD was expressed this was not able to detect the virus, Further processing may be developed for purification of BTV VP7 UD.

Conclusions:-

In conclusion, the BTV VP7 upper domain protein was expressed in pQE-30 vector; but it was very difficult to purify as the VP7 protein is an insoluble protein. So further studies will carried to express and purification of BTV VP7-UD as it is having surface epitomes and conserved among all known serotypes.

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