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Research Article

RT-PCR AMPLIFICATION OF G GENE OF CHANDIPURA AND VESICULAR STOMATITIS NEW JERSEY VIRUSES AND CLONING IN pTARGET VECTOR

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ABSTRACT
RT-PCR amplification of VSV G gene was done successfully and same was cloned in pTarget vector.

Keywords: RT-PCR, G gene, Chandipura virus, vesicular stomatitis New Jersey virus, gene cloning, cDNA.

Work done at Microbiology Laboratory, Department of Pathology, College of Veterinary Medicine and Biomedical sciences, Colorado State University, Fort Collins, Colorado, USA under FAO Fellowship in 1998.

INTRODUCTION

Vesicular stomatitis is an important livestock disease endemic in the Americas. It is a zoonotic viral disease characterized by vesicles, erosions and ulcers on the mouth, feet and udder. Pain, anorexia and secondary mastitis can cause decreased productivity in all species, and swine infected with some viruses may die. Although vesicular stomatitis is endemic in limited areas of the South Eastern United States, these viruses have not caused outbreaks in livestock since the 1970s. Vesicular stomatitis closely resembles three vesicular diseases: foot-and-mouth disease (FMD), swine vesicular disease, and vesicular exanthema of swine.

Vesicular stomatitis virus (VSV) is a member of the genus *Vesiculovirus* in the family Rhabdoviridae (Acha and Szyfres, 2003., Krauss *et al*, 2003). VSV is a bullet-shaped, enveloped virus, approximately 70 nm in diameter and 170 nm in length, and has a single-stranded, negative-sense RNA genome (Rodríguez, 2002., Lichty *et al*, 2004). VSV has eight main serotypes: Indiana, New Jersey, Cocal, Alagoas, Isfahan, Chandipura, Maraba, and Piry. Most human infections with Indiana and New Jersey VSV serotypes appear to be subclinical (Letchworth *et al*., 1999). In patients that show clinical manifestations, the initial symptom is high fever that is often biphasic. Subsequent symptoms are "flu-like" including severe malaise, headaches, myalgia, arthralgia, retrosternal pain, eye aches, and nausea. Chandipura

virus has only been reported in India, where it mostly infects children. Symptoms include fever, sensory disorders, convulsions, vomiting, diarrhoea, and encephalitis leading to coma and death (Mullen and Durden, 2009). The two major serotypes are New Jersey and Indiana. Currently, four viruses are known to cause vesicular stomatitis: vesicular stomatitis Indiana virus (VSV-IN), vesicular stomatitis New Jersey virus (VSV-NJ), vesicular stomatitis Alagoas virus (VSV-AV) and Cocal virus. Vesicular stomatitis mainly affects horses, donkeys, mules, cattle and swine. South American camelids, sheep and goats occasionally have clinical signs. Serological evidence of infection has been found in many other animals including deer, pronghorn antelope, bighorn sheep, bats, raccoons, opossums, lynx, bobcats, bears, coyotes, foxes, dogs, non-human primates, rabbits, rodents, turkeys and ducks. Humans are also susceptible.

The present work was undertaken for gene amplification and its cloning to pursue further work using such an important virus.

MATERIALD AND METHODS

Isolation of viral RNA

It was done from virus infected cell culture fluid available in the laboratory using QIAamp viral RNA kit (QIAGEN) using kit protocol.

Synthesis of first strand cDNA coding glycoprotein G

cDNA was prepared using cDNA cycle kit of Invitrogen and lab protocol. Prepared 50µl reaction mix containing 5µl of 0.1M DTTY, 10 µl of 5X 1st strand buffer, 2.5 µl of 10mM each dNTPs, 1µl of Gi oligo forward primer (0.6µg/µl), 1µl RNasin, and 20.5µl DEPC water. Mixed the solution, added 10µl viral RNA extracted earlier, vortexed, spun down and then kept at 65^{o}C for 5 min in a water bath. Annealed the primer for 10 min at room temperature and then added 2.5µl MLV reverse transcriptase (200U/µl), 1 µl RNasin (10-40 U) and incubated at 37^{o}C for 90 min. Then put it at 92^{o}C for 2 min to denature RNA-cDNA hybrid and then quickly placed the tube on ice. Then set up PCR.

PCR

PCR was done using Invitrogen cDNA cycle kit and lab protocol. Prepared 100µl PCR reaction mix which contained 72.5µl distilled water, 10µl of 10X PCR buffer, 10µl MgCl2, 1µl dNTPs, 2µl primer F, 2µl primer R, 0.5 µl Taq DNA polymerase and then mixed , spun, added oil and added 2µl first strand viral c-DNA and again spun. Put the tube in Perkin Elmer DNA Thermal Cycler using the cycling conditions: 35 cycles wherein each cycle consisted of 94^{o}C for 1 min, 57^{o}C for 1.5 min 72^{o}C for 2 min with a final extension at 72^{o}C for 10 min and finally hold at 4^{o}C. A 600 bp G fragment of VSV NJ was also amplified using its specific F and R primers using these conditions.

Agarose gel electrophoresis was done using 0.9% agarose in 1X TAE buffer containing 0.5µg/ml EtBr (Sambrook *et al*, 1989). The sample volume was 10 µl containing 5 µl PCR product, 4 µl distilled water, 1 µl 10X loading buffer, mixed spun and loaded. The electrophoresis was initially run at 50 V for few minutes and at 100V using BioRad electrophoresis equipment.

Cloning of G gene

The G gene was cloned in pTargeT vector using ligation and transformation using Original TA Cloning Kit protocol of Invitrogen except that mercaptoethanol was not used.

Plasmid amplification

Three ml of LB broth containing 100 µg/ml ampicillin in each tube was inoculated with *E.coli* DH5 alpha containing the rplasmid using 3 µl/tube, kept in shaking incubator at 37^0C, 200 rpm overnight.

Plasmid DNA isolation

The culture tubes were processed for Miniprep plasmid DNA purification using Wizard plus SV Miniprep DNA Purification System kit protocol (Promega). The DNA isolated was read in Beckman DU-70 spectrophotometer at $A_{260/280}$ after diluting the DNA 1/50. The agarose electrophoresis was done using agarose concentration of 0.7 or 1% as per requirement.

The RE digestion of rplasmid DNA was done using the reaction mix of 10µl containing 2µl plasmid DNA, 1µl 10X buffer, 1µl RE and 6 µl distilled water and then incubated at 37^0 C in an incubator for 2 hrs.

The RE digestion of rplasmid DNA for isolation of gene insert was done using the reaction mix of 50µl containing 15µl plasmid DNA (VSV NJ), 5 µl 10X buffer, 5 µl BSA, 2µl *Sal*I, 2µl *Xho*I and 21µl distilled water and then incubated at 37^0 C in an incubator for 4 hrs. Agarose gel electrophoresis was done in two parallel lanes, one was loaded with 50µl containing 45µl DNA digest with 5µl loading buffer and another lane with 10µl containing 5µl DNA digest with 1µl loading buffer and 4µl distilled water. Gels were photographed in Gel Documentation System IS-1000 Digital Imaging System (Seikosha VP-1500).

Colony PCR

Picked one white transformed colony and streaked on a LB agar plate with appropriate antibiotic. Then dipped the tip in distilled water in a microfuge tube and mixed by pressing the micropipette push button. Then took 10µl from this microfuge tube and put the microtip into a LB broth tube with appropriate antibiotic. LB agar plate and LB broth tube are incubated at 37^oC for growth of culture.

For colony PCR, took the microfuge tube with distilled water and the inoculums and boiled for 15 min, then kept on ice for 10 min and the immediately set up the PCR using gene specific F and R primers. The DNA bands were isolated from the gel using QIAquick Gel Extraction kit protocol (QIAgen).

RESULTS AND DISCUSSION

The results of RT-PCR of Chandipura virus and VSV NJ virus glycoprotein G gene are shown in Fig 1&2 which revealed that lab protocol yielded good results. The amplified genes were successfully cloned in pTargeT mammalian expression vector and digestion of the rplasmid with appropriate restriction enzyme released the cloned insert.

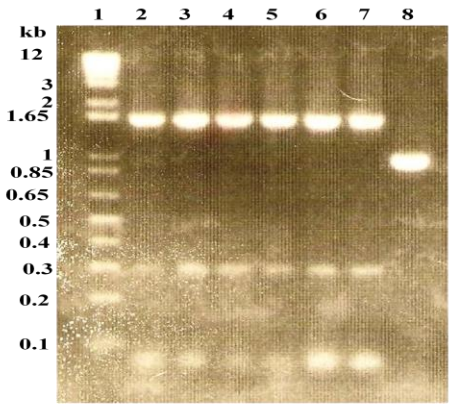

Fig.1. Amplification of cDNA
Lanes1-1kb plus DNA ladder,
2-MS2 RNA Invitrogen kit and protocol
3-CN RNA Invitrogen kit and protocol
4-CN RNA Invitrogen kit and lab protocol
5- MS2 RNA Invitrogen kit and lab protocol
6- CN RNA Invitrogen kit and lab protocol
7-Lab protocol reagents and lab protocol
8- CN virus G gene cDNA

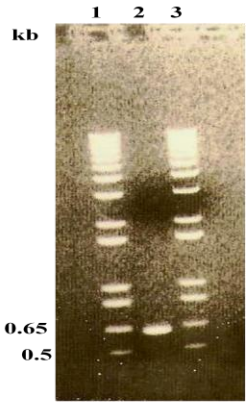

Fig.2. Amplification of cDNA
Lanes 1,3 - 1kb plus DNA ladder
2- VSV NJ G gene 0.6 kb fragment purified by QIAgen PCR purification kit

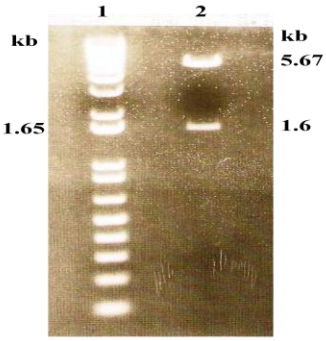

Fig.3. Digestion of pTargeT r-DNA with XhoI and SalI.
Lanes 1-1kb plus DNA ladder, 2-Release of 5.67kb pTargeT DNA and 1.6 kb VSV NJ G gene insert..

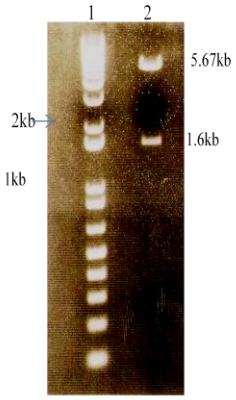

Fig 4. pTargeT rDNA digested with XhoI + SalI showing pTargeT 5.67 kb and VSV NJ G 1.6 kb insert

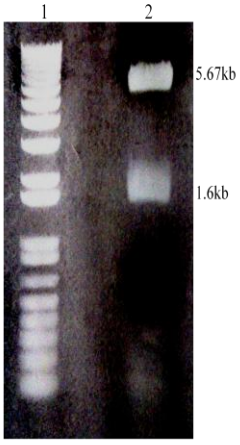

Fig 5. pTargeT rDNA digested with XhoI + SalI showing pTargT 5.67kb and VSV NJ G 1.6kb insert for extraction.

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