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Large Scale of Rabies Glycoprotein G Gene Grow in *E.coli* using Fermentor which can be use as a Vaccine

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Abstract

Rabies is preventable viral zoonotic (Animal born) neuroinvassive disease (i.e infecting the central nervous system).In the present study, the bacterial culture of *E.coli* (DH5α) strain containing recombinant plasmid (Rabies glycoprotein G gene) was amplified in L.B medium containing antibiotic ampicillin(100µg/ml). Plasmid DNA isolation was carried out for every sample and then was confirmed on 1% Agarose gel electrophoresis for enumeration of success of fermentation process. Many different bands are seen of DNA by different interval time. Hence the recombinant plasmid can be used as DNA vaccine in canines against rabies.

Keywords: Rabies virus (Glycoprotein G gene), *E.coli* strain (DH5α), L.B. Medium, Ampicillin.

Introduction

Rabies is preventable viral zoonotic (Animal born) neuroinvassive disease (i.e infect the central nervous system). The word Rabies originates, from Sanskrit rahbas meaning "to do violence". The Greek culture had two gods of rabies. Aristaeus who counteracted the effect and Artemis who was the healer of rabies.Rabies was first reported in 1709 at Mexico (America).The first epidemics of rabies in North America

were described in 1770-1771. The rabies virus was finally isolated in 1885 by Dr. Louis Pasteur ,he then produced the first rabies vaccine by inactivating virus using ultra-violet radiations. Modification of his vaccines are still used today.It is transmitted through the bite of rabid animal. World-wide most cases arise from dogs. Rabies is prevalent in Latin America, Asia and Africa. Dogs are reservoir of rabies virus and dog to dog transmission occurs very rapidly. Over the last hundred years, rabies in the united states has changed dramatically. More than 90% of all animal' cases reported annually to CDC (Centre for Disease control and Prevention). Before 1960, the majority were in domestic animal. The principle rabies host was wild carnivores and bats, presently exposure to rabid dogs is the cause of more than 99% of human rabies death world-wide. *(Smith and Siedel,1993).* Rabies virus is a negative stranded RNA virus of Lyssavirus genus in the family Rhabdoviridae. Although convention vaccines againt rabies are available. These are not ideal for mass vaccination in developing countries like India. Out five proteins encodes by rabies genome, glycoprotein is the only protein capable of inducing and reacting to virus neutralizing antibodies (VNA) and for conferring protective immunity against lethal challenge with rabies This has laid down the possibility of developing a subunit vaccine against rabies. DNA vaccines against have been reported and found to be successful as per-exposure vaccination in mice (Perrin *et al*., 2002; Lodmell and Gwalt, 2001., Rai *et al.,* 2002 Lodmell *et al.,* 2002), dogs (Forg *et al.,* 1998), cats (Akbari *et al.,* 1998), and non human primates (Krieg, 2000), Further, over expression of rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response (Faber *et al.,* 2002).

Materials & Methods

Vector

NBC, Indian Vetenary Research Institute, Izatnagar, Bareilly (U.P) provided a replicase based mammalion the recombinant plasmid was transformed in Escherichia coli DH5α cell and maintains in L.B medium containing 100µg/ml ampicillin.

Host Bacterial Strain

Escherichia Coli (DH5α) (Promega, Madison) host strain were used for transformation with recombinant plasmid.

Standard markers

DNA molecular weight markers used was 1KB DNA ladder (MBI Fermentas, MD).

Chemicals and Plastic wares

All chemicals used in the study were either MBI Fermentas (MD), New England Biolabs (MA), Banglore Genei (Banglore,India) Plastic were and other consumables are from Axygen. TRP nune.Greiner, Torsons, corning and Borosil.

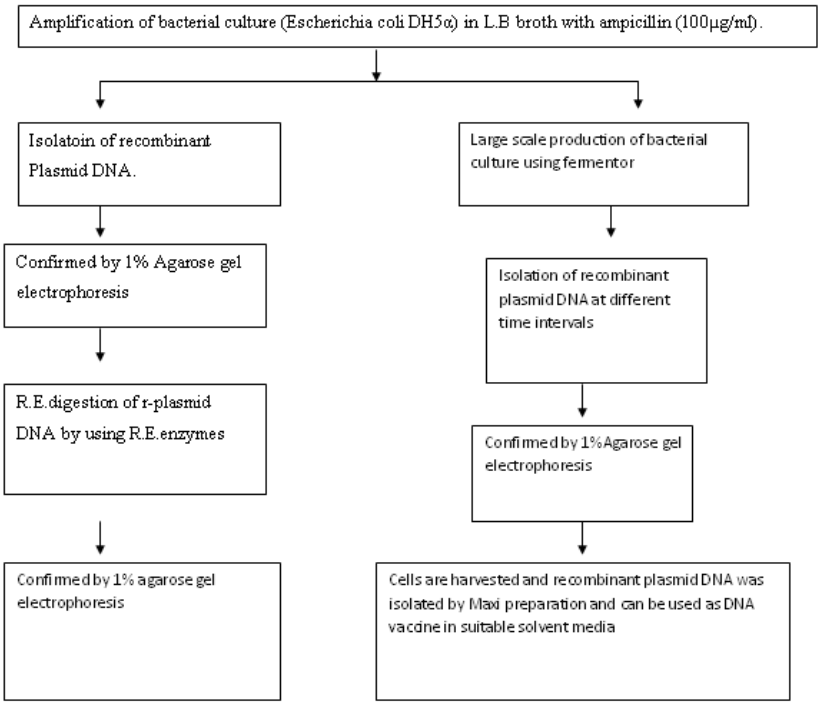

Figure 1: Flow diagram showing methodology of present work.

Isolation of Plasmid DNA

Small- scale plasmid DNA isolation was done following the alkali lysis methed (Birnboin and Dolly, 1979). Briefly, individual colonies were inoculated in 5ml L.B broth with appropriate antibiotics and were kept in orbital shaking incubator overnight. 3 ml of overnight grown broth culture taken in eppendorf tube. Spun for 30 sec at 12000 rpm, discard supernatant, take pellet. Resuspended the pellet in 300µl of buffer P1 by vortexing. Then added 300µl of buffer P2, mixed gently and incubated at room temperature for 5 minutes. Spun for 10 minutes at 12000rpm, transferred the supernatant to a new microfuge tube. Supernatant was extracted with phenol: chloroform: isoamyl alcohol (25:24:1 v/v), Mixed vigorously and spun for 15 minutes at 12000rpm. The upper aqueous phase containing the plasmid DNA was collected and precipitated with 0.8 volume of isopropanol, spun at room temperature for 30 minutes at 12000rpm, supernatant was discarded. Pellet was washed with 1ml 70% ethanol at 12000rpm for 1 minutes. Air dried and eluted with 40µl of T.E buffer. Electrophoresis and photographed on geldoc.

Restriction endnuclease digestion

The plasmid DNA was incubated for a particular time period with appropriate 1X restriction endonuclease buffer and restriction endonuclease at 37^0C in water bath. The digested DNA was mixed with 1X loading dye and 1% agarose (SRL, Mumbai) gel using tris acetate EDTA(TAE) running buffer system containing ethidium bromide(0.5µg/ml) and visualized under UV-transilluminater.

Digestion with EcoRI enzyme requires:

Plasmid DNA	3µl
Enzyme	1µl
Buffer	1.5µl
D.W.	9.5µl
Total	15 µl

Digestion with BamHI enzyme requires:-

Plasmid DNA	3µl
Enzyme	1µl
Buffer	1.5µl
D.W.	9.5
Total	15 µl

Mixed and incubated at 37^0C in water bath for 3-4 hours and runned on gel and visualized on Geldoc and photographed.

Agarose Gel Electrophoresis

Agarose, type 1. Gelling temperature (40-42^0C) from SRL (Mumbai) was used to perform majority of gel electrophoresis in concentration of 0.8% to1.2% for separation of DNA fragments ranging from 0.4Kb to10Kb. Appropriate amount of agarose was mixed with 1XTAE buffer and melted in microwave oven. When the molten gel had cooled to about 42^0C. Ethidium bromide was added to final concentration of 0.5µg/ml. The gel was mixed thoroughly by gentle swirling and then poured into the gel casting tray fitted with the comb. The gel was allowed to solidify and the comb was removed.

The DNA sample were mixed with gel loading dye 1X (Final concentration) and loaded onto the wells. The gel was run at a voltage of 1.5V/cm for 1-2 hours and the hands were visualized onder UV light and photographed on geldoc.

Fermentation Process

Primary inoculums preparation

100ml L.B broth with 250µl ampicillin and 200µl culture. Incubated at 37^0C for overnight in orbital shaker at 200rpm. One day before the process.

Media preparation for fermentation

Five litres of L.B broth is prepared with addition of 5ml ampicillin and 100ml primary inoculum as prepared one day earlier.

Working

Fermentation process started at 8.20 A.M. Having following parameters.

a. Impellar speed =150rpm

b. Air pressure =1 pound/inch
c. Air flow rate =(1).2 litre/min up to 2hrs. After initiation of process.
(2).Up to 3 litre/min 2^{nd} to 4^{th} hours.
(3).Up to 5 litre/min from 4^{th} hour until Last
d. Dissolved oxygen =0.01%
e. Temperature =37^0C

Sample at regular intervals are taken of which:
O.D:- (600nm), pH:- DNA presence is estimated after isolation.

Time at which sample are withdraw:
8:20 AM
10:20 AM (2hrs)
12:20 AM (4hrs)
2:20 PM (6hrs)
4:20 PM (8hrs)
5:00 PM (8hrs.40min)
Total Time span = 8hours 40 minutes.

Harvesting of transformed E.coli cells (DH5α):
The bacterial culture transferred into different bottle capacity 500ml.then pelleted by centrifugation at 5000rpm for 15 minuts.after that Supernatant discard and stored at -20^0C for overnight.

Isolation of Plasmid DNA from transformed *E.coli* cells (DH5α) harvested after fermentation:

Transform E.coli cells were subjected to plasmid isolatin using first three step of Alkali-lysis methed (Birnboim and Dolly1979). The result was then filtered using filter paper (Sartoriour Germany) and then filter was through silica Guanidine columns from isolation of recombinant plasmid DNA eluted in PBS/NSS and can be used as a DNA vaccine.

Result

Overnight grown *E.coli* DH5α bacterial culture in L.B. medium having ampicillin (100μg/ml) showed visible growth as the culture was turbid.

Isolation of recombinant plasmid DNA:

Bacterial culture subjected to plasmid DNA isolation using alkali lysis methed. Plasmid DNA thus isolated when analysed 1% Agarose gel electrophoresis showed clear band of expected size (Super coiling DNA).(Fig.2)

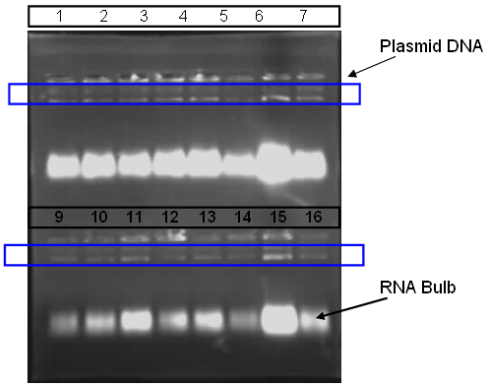

Figure 2: 1% Agarose Gel electrophoresis of recombinant plasmid DNA Lane 1 to 16: - Isolated recombinant Plasmid DNA.

R.E. analysis of recombinant Plasmid DNA

R.E digestion of recombinant plasmid DNA using EcoRI and BamHI with their specific assay. Buffer gave clear band by expected size this R.E digestion of plasmid DNA (EcoRI) produced fragment of 5.9Kb size while DNA fragment produced by BamHI was 5.5Kb size. (Fig.3 & fig.4).

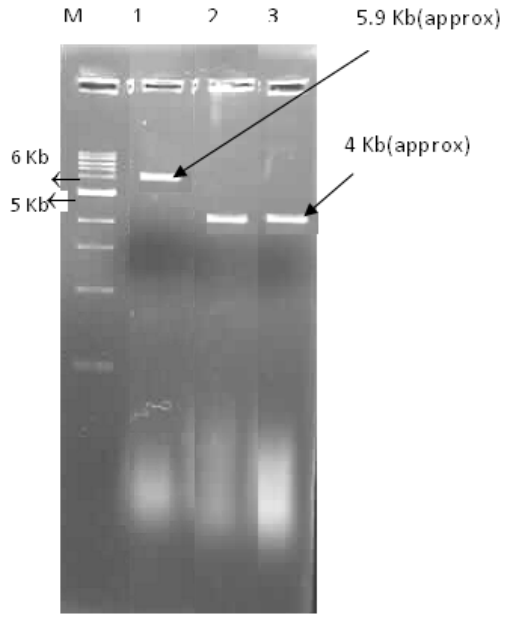

Figure 3: 1% Agarose Gel electrophoresis of recombinant plasmid DNA digested with EcoRI **Lane M** - 1 Kb DNA ladder
Lane 1 - Plasmid DNA digested with EcoRI **Lane 2 & 3** - Undigested Plasmid DNA.

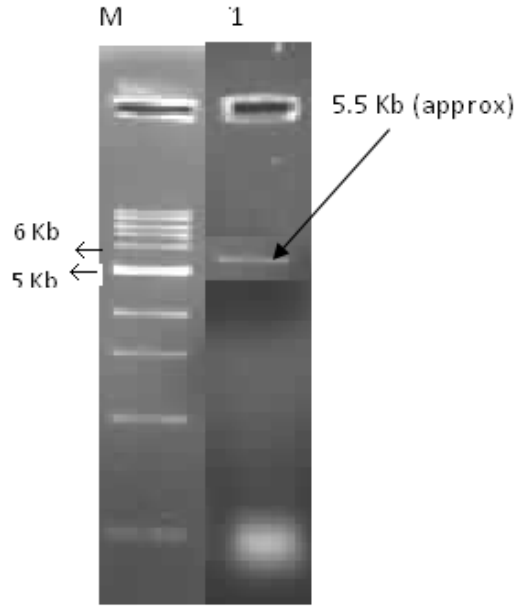

Figure 4: 1% Agarose Gel electrophoresis of recombinant plasmid DNA digested with Bam HI **Lane M** - 1 Kb DNA ladder **Lane 1** - Plasmid DNA digested with Bam HI

Large scale production of transformed E.Coli (DH5α) cells using fermentor
On the conformation by 1% Agarose gel electrophoresis it was observed that for the production of high content of DNA the culture should be grown at 8 to 10 hours in the fermentor at optimum condition (fig .5).

Table 1: Quality and Quantity of DNA at different time intervals.

No. of Sample	Time of Sampling	O.D	(pH)	D N A band
1.	0 hrs.	0.04	7.2	No band
2.	2 hrs.	0.08	6.6	Very Light Band
3.	4 hrs.	0.44	6.0	Very Light Band
4.	6 hrs.	0.95	6.0	Light Band
5.	8 hrs.	1.5	6.0	Thick Band
6.	8 hrs. 40 min.	>1.5= 2.0	>6.0	Very Thick Band

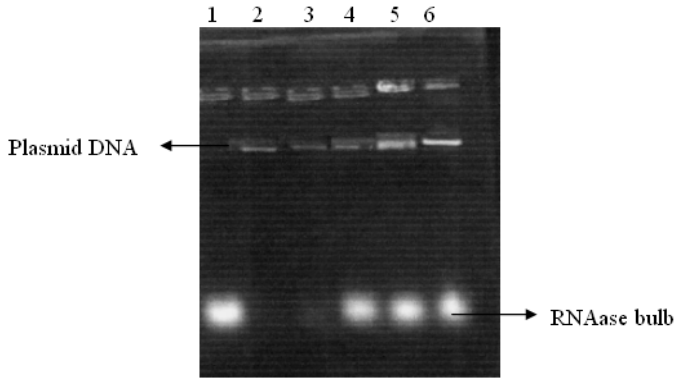

Figure 5: Gel showing plasmid DNA isolated sample collected during the process of fermentation at different time intervals.
Line1- At 0 hour, No band observed
Line2- At 2 hours very light band are seen
Line3- At 4 hours very light bands are seen
Line4- At 6 hours light band are seen
Line5- At 8hours thick band are seen
Line6- At8 hours-40 minute very thick band are seen.

Discussion

During the experiment the O.D of plasmid DNA was observed on the base of different interval times when increase the time O.D. is also increase. In the first step time is 0 then saw the O.D was 0.04after after that increasing time 0 to (8 hours 40 minuts) then optimize O.D was 2.0.and on the bases of time and O.D. then DNA also show the thick and clear bands. The quality and quantity of the plasmid DNA was very

good. I have also tried to grow the recombinant *E. coli* for the plasmid DNA using L.B media to make the vaccine.

Conclusion

The above experiment shows that it is possible to produce vaccine in L.B.medium.We can be use this experiment for production in vaccine against the diseases and this provide humoral as well as cell mediated immunity. This type we can save the life of the mammal by preparing DNA vaccine.

Acknowledgement

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