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CLONING AND EXPRESSION OF 3CD GENES OF FMDV

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SUMMARY: RT-PCR amplification of VIA (3D) gene and protease (3C) using its primers. Legation of the PCR product with the cloning vector (using pET system). Expression of the recombinant plasmid in E. coli expression host. Use of the expressed VIA protein in diagnosis of cattle infected with any of the 7 serotypes of FMD virus with the help of ELISA.

INTRODUCTION

Assays which detect antibodies to non-structural proteins of foot and mouth disease virus (FMDV) have the potential to differentiate animals which have been infected with the virus from those that have been vaccinated (Neitzart et al., 1991; Bergman et al., 1993). A non structural antigen associated with FMDV replication, the virus infection associated antigen (VIAA) (Cowan and graves, 1966), is composed mainly of the RNA polymerase, designated as 3D protein. The antigenicity of the protein is conserved amongst all serotypes. Our aim was the application of biotechnology to produce recombinant FMDV polymerase for use in ELISA as antigen for detection of antibodies that specifically indicate a postinfection state (O' Donnell et al., 1996).

MATERIAL AND METHODS

Preparation of recombinant proteins.

The sequence corresponding to the 3CD region of FMDV type O was amplified by PCR and cloned into pET 21 a-d vector (Fig No 1) and transformed to expression host induced by IPTG to express the protein of the inserted sequence.

The molecular weight of the final products were confirmed by polyacrylamid gel electrophoresis (PAGE) and by liquid phase blocking sand wich ELISA using two reference polyclonal antibodies (O' Donnell et al., 1996).

ELISA:

Indirect ELISA was developed for the detection of antibody to FMDV 3D in cattle sera. Coating of ELISA plate with antigen using carbonate bicarbonate buffer, pH 9.6 by overnight incubation at 4°C, then washing with phosphate buffered saline containing 0.05% tween 20, nonspecific protein binding sites were blocked by incubation for one hour at 37°C, blocking buffer (1 % lactalbumin in PBS with tween 20 0.05%) 50 µl per well, 50µl of each test serum was added to two wells and the plates were incubated for a further hour at 37°C. Gunea pig reference hyperimmune serum was added to each well the same time negative control with negative serum was made, incubated for one hour, conjugate was added anti Ginea pig conjugated with horse radish peroxidase enzyme for a further hour at 37°C. Finally, the reaction was developed using 50µl per well orthophenyldiamine (OPD) and 0.005 % H2O2. The reaction was stopped after 15 minutes by addition of 50 µl per well 1.25 M H2SO4 and the absorbance of wells was read at 492 nm.

Sera:

Negative sera were collected from 40 animaals from FMD free area which were negative for antibody to FMDV either by serum neutralization test or ELISA. Positive sera were collected from 5 experimentally infected calves, sera collected 7 day postinfection until 12 weeks.

RESULTS

Serial double fold dilutions of the bacterial lysate containing the expressed proteins (the antigen) were prepared to standardize and estimate the optimal dilution which would gave positive results against reference VIA positive hyperimmune serum. The optimal dilution was 1/320. Negative control (blank) O. D. 0.2 positive control O. D> 0.6 this was shown in Fig No (2).

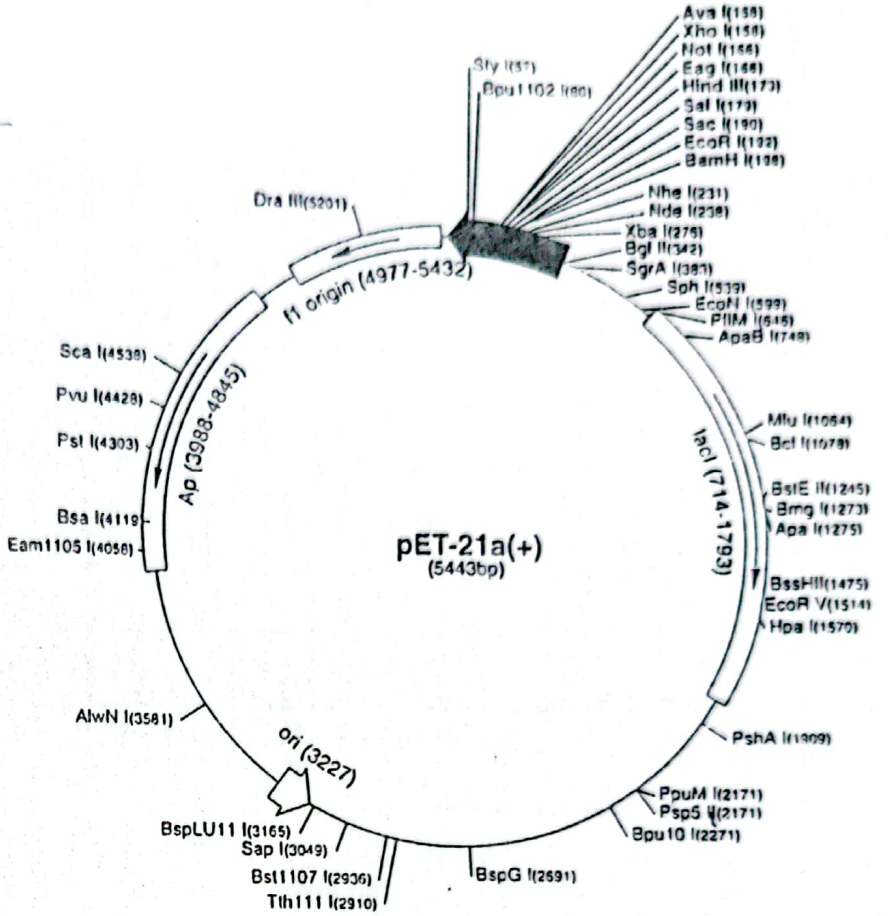
Coating of ELISA plate with this dilution of antigen, all negative cattle sera gave negative results by ELISA-3D at 1/320 concentration.

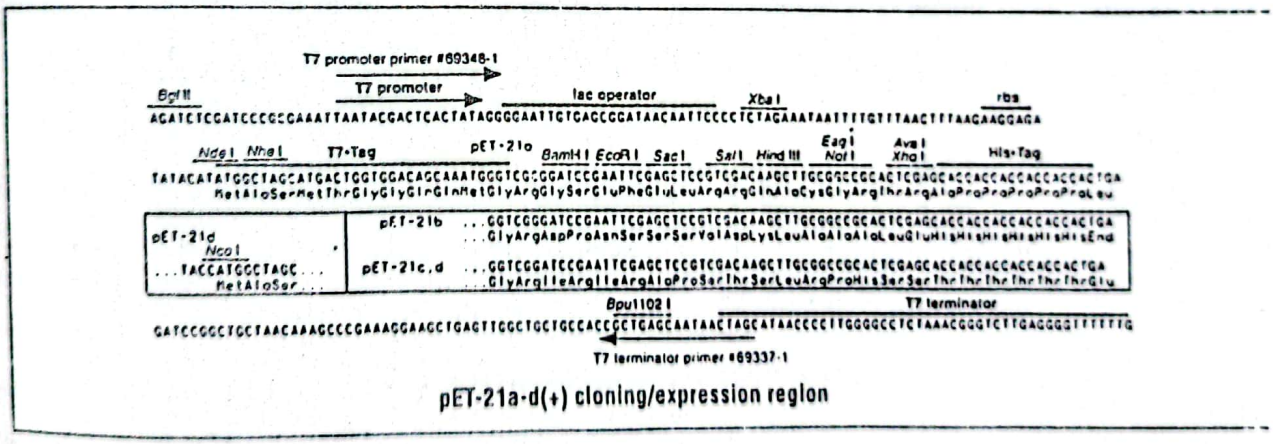
Antibodies to the 3D protein were detected from 7 day post infectioa.

Further samples become positive in all animals

pET-21a-d(+) Vector

pET-21a(+) sequence landmarks	
T7 promoter	311-327
T7 transcription start	310
T7•Tag coding sequence	207-239
Multiple cloning sites (*BamH I - Xho I*)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	714-1793
pBR322 origin	3227
bla coding sequence	3988-4845
f1 origin	4977-5432





pET-21a-d(+) cloning/expression region

Figure No. (1) pET -21 a-d

Table 1: Detection of VIA antibodies in sera samples collected weekly intervals from experimentally infected calves.

Weeks post infection	Animals				
	1	2	3	4	5
0	0**	0	0	0	0
0	0	0	0	0	0
1	+*	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	+	+	+
12	+	+	+	+	+

VIA antibodies could be detected in sera samples at 7 days post infection.
* Sample contained antibodies against VIA antigen.
** Sample did not contain antibodies against VIA antigen.

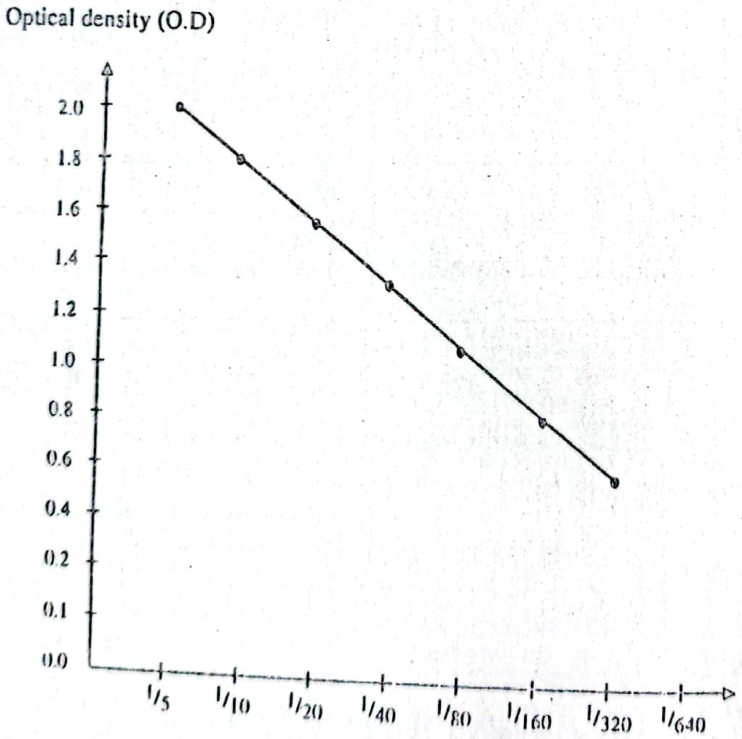


Figure No. (2): Serial double fold dilutions of the bacterial lysate containing the expressed proteins (the antigen) to standerdize and estimate the optimal dilution against reference VIA positive hyperimmune serum.
N.B.: The optimal diluton was 1/320
Negative control (blank) O. D. 0.2
Positive control O.D≥ 0.6.

until 3 months this was shown in table No (1).

DISCUSSION

Diagnosis of FMD in infected animals relied up on for several decades on the detection of antibodies developed against the VIA antigen of FMDV which is encoded by the polymerase gene (3D) region. For this reason several attempts have been carried out to extract and purify the VIA antigen from FMDV infected cells, to be used (Alonso et al., 1990).

The antigenic specificity of expressed proteins have been tested by using sandwich ELISA with help of two reference hyperimmune serum to FMDV. Serial double fold dilutions of the bacterial lysate containing the expressed protein (the antigen) have been made to standardize and estimate the optimal dilution of this antigen against reference hyperimmune serum to VIA to be used for coating of ELISA plates (this optimal dilution was 1/320, (Fig No. 2).

Coating of ELISA plate with this diluted antigen could provide us with a patent tool for determination of antibodies to FMDV VIA antigen in infected animals. The test proved its specificity based on negative reaction given by sera collected from non infected animals (table No. 1).

Generally speaking, the non structural proteins has been used by several workers as diagnostic antigens (Cowan and Graves 1966; McVicar and Sutmoller 1970; Centeno et al., 1979), but it is very interesting to note here that these above mentioned authors have been working with the non structural proteins including the VIA antigen prepared by the convential methods.

In conclusion, the application of biotechnology and genetic engineering in the production of recombinant FMD polymerase antigen (3D) could overcome the problem of creating a consistent source of diagnostic antigen. In addtion, the use of non infectious material in the production of this protein could help greatly in preventing the spread of FMDV to environment. Beside this, the use of virulent virus need special laboratory containment devices.

And finally, the method and devices used in cloning and high expression of 3C and 3D proteins provided us with a powerful tool for the large scale production of the VIA antigen (3D) to be used as diagnostic tool for detection of antibodies against FMDV in naturally and persistently infected animals.

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