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ONE-STEP RT-PCR TARGETING THE FUSION PROTEIN GENE FOR DETECTION OF BOVINE RESPIRATORY SYNCYTIAL VIRUS

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SUMMARY

RT-PCR F targeting a fragment of the fusion protein (F) gene of bovine respiratory syncytial virus (BRSV) has been recommended to detect BRSV. This assay is used to be performed in two steps after RNA extraction: cDNA synthesis then PCR. In this study, cDNA synthesis and PCR have been performed in one step single tube. This modification may save time and effort with the same sensitivity and specificity.

INTRODUCTION

Bovine respiratory syncytial virus (BRSV) isolation is difficult because of its lability (Smith et al., 1974). PCR assays may be more sensitive than immunofluorescence (IF) (Vilcek et al., 1994; Valarcher et al., 1999) and ELISA (Larsen et al., 1999; Valarcher et al., 1999) and as specific as IF and ELISA (Valarcher et al., 1999) for the detection of BRSV infections. RT-PCR F targeting the fusion prctein (F) gene has been recommended for detection of BRSV (Eleraky et al., 2003). In the current study, modification in the previous assay (Eleraky et al., 2003) has been performed in order to save time and effort. Also, the sensitivity and specificity of the assay after modification has been evaluated.

MATERIAL AND METHODS

RNA extraction:

Trizol L S (Invitrogen Life Technologies) was used to extract the RNA from the viral suspensions according to the manufacturer'

s protocols. The virus used was BRSV 391-2 (Provided by Dr. Gail Wertz, Dept. of Microbiology, Univ. of Alabama Medical School, Birmingham, AL). The RNA pellet then was air dried and

dissolved in Rnase-free water. The RNA concentration was quantified in a spectrophotometer at A260 wavelength.

RT-PCR:

Superscript One-Step RT-PCR with Platinum Taq (Invitrogen Life Technologies) was used to perform cDNA and PCR in a one-step single tube to amplify 426 bp of F gene of BRSV. The followings were added in one PCR tube per 50 ul reaction volume: 25 ul 2X Reaction Mix, 1 ul F103 primer (10 uM), 1 ul F493 primer (10uM), 1 ul RT/Platinum Taq Mix, 0.04 - 0.4 ug RNA and Rnas-free water up to 50 ul. The F103 primer (5'TGCCAAAACATAACAGAAG 3') and F493 primer (5' TTTATTCACCTCTCCCTC 3') were used in a previous study (Eleraky et al., 2001). The following cycling conditions: 1 cycle of 45°C for 30 minutes (for cDNA synthesis), 1 cycle of 94°C for 2 minutes (for denaturation), then a three-step cycling program (repeated 40 times) consisting of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds. Final extension step is performed at 72°C for 10 minutes.

Sequencing:

Sequencing of the PCR product was done at the University of Tennessee Molecular Biology Research Facility by using an ABI prism dye terminator cycle sequencing reaction kit and an ABI 373 DNA sequencer, Perkin Elmer Inc., Foster City, CA. Then, electrophoresis was done in three percent high-resolution agarose (Sigma Chemicals Co., St. Louis, MO).

Virus strains:

In addition to BRSV 391-2 isolate, the assay was evaluated with the following BRSV isolates (375, FS1-1, NMK7, MN, CA, NY191285, NY55798, NY586127, NY237424, NY414458, NY561388, 1143, 1144, 1156R, MD5190, CO16186 and TN). These isolates were kindly provided by Dr. John Baker, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan., Dr. James Collins, Colorado State University, Fort Collins, CO, and Dr. John Black, American Bioresearch Inc., Seymour, Tennessee, USA.

Also, several other viruses associated with bovine respiratory tract disease (bovine herpesvirus 1, bovine parainfluenza 3, bovine virus diarrhea virus, bovine coronavirus and bovine adenovirus 3) were included in the study. These isolates were provided by the Clinical Virology Laboratory, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA.

Cell lines:

MDBK line cells were used for growing of the different isolates of BRSV, bovine herpesvirus 1, bovine parainfluenza 3, bovine virus diarrhea virus and bovine adenovirus 3. HRT line cells were used for growing of bovine coronavirus.

RESULTS

Using one-step RT-PCR resulted in amplification of a 426 bp with BRSV strain 391-2 (Fig. 1). The nucleotide sequence of the amplified product was compared with the corresponding sequence in the Gene Bank. Nearly complete identity was shown between the amplified product in this study and the published sequence (Lerch et al., 1991) (Gene Bank accession number M58350) of the same virus strain (Fig. 2). All the other seventeen BRSV isolates tested were successfully amplified giving the same product.

No PCR products resulted in with other viruses associated with respiratory manifestations (bovine herpesvirus 1, bovine parainfluenza virus 3, bovine virus diarrhea virus, bovine coronavirus and bovine adenovirus 3).

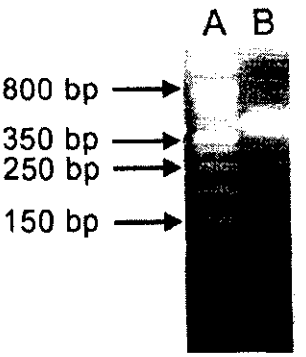

Figure 1: Gel electrophoresis of the PCR F product obtained from BRSV strain 391-2. Lane A contains the 50 bp DNA ladder. Lane B represents the PCR product (426 bp).

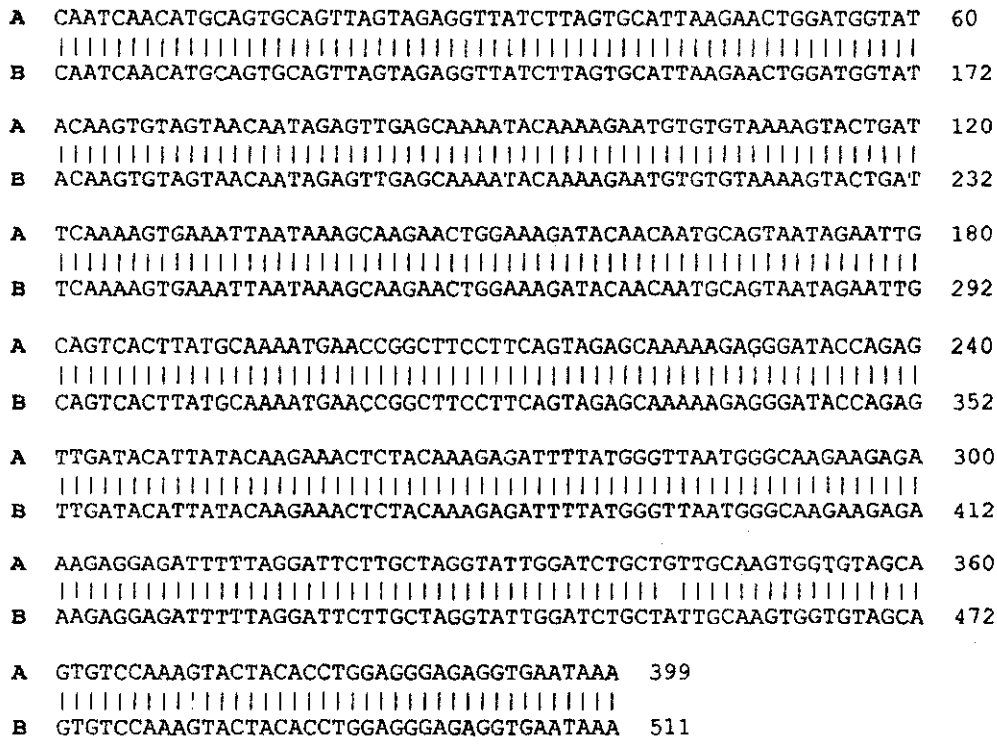

Figure 2: The nucleotide sequence of the amplified PCR F product of BRSV strain 391-2 (A) compared to the published sequence (B) of the same strain (Gene Bank accession number M58350). Each line represents 60 nucleotides. The number of the last nucleotide for each line is given on the left end of the line.

DISCUSSION

One-step single tube RT-PCR could detect the eighteen isolates of bovine RSV. Therefore, it proved as sensitive as the method described before using two steps; reverse transcription then PCR (Eleraky et al., 2001; Eleraky et al., 2003). Identity between the nucleotide sequence of the amplified product in this study and the corresponding published sequence sequence (Lerch et al., 19991) in the Gene Bank confirmed the fidelity of this assay.

Also, no PCR product was obtained with other viruses associated with bovine respiratory tract disease (bovine herpesvirus 1, bovine parainfluenza virus 3, bovine virus diarrhea virus, bovine coronavirus or bovine adenovirus 3). This indicates that this assay is as specific as the previous assay (Eleraky et al., 2001; Eleraky et al., 2003).

Moreover, it can save time and effort. Therefore, this modification is useful to facilitate identification of BRSV.

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إستخدام RT-PCR (الواحدة) فى تشخيص BVSV

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إختبار تفاعل البلمرة التسلسلى (PCR) يعتبر من الإختبارات الحساسة والفعالة لتشخيص كثير من الڤيروسات التى تصيب الحيوانات ومنها ڤيروس BRSV.

فى هذا البحث إستخدم إختبار RT PCR (الخطوة الواحدة) الذى إستهدف الجين الخاص ببروتين الإلتحام (F protein) فى تشخيص المعزولات المختلفة من BRSV بنجاح وحساسية جيدة.