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Rapid Detection of Sacbrood Virus (SBV) by One-Step RT-PCR Assay

Ke-Fei Shen, Lan-Cao and Jin-Long Yang
Chongqing Academy of Animal Science, 402460 Chongqing, China

Abstract: A one-step Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay was developed for the rapid identification of Sacbrood Virus (SBV). The data demonstrated: The one-step RT-PCR method is fast and useful for detecting the honeybee virus.

Key words: Sacbrood virus, RT-PCR, detect, honeybee, virus, assay

INTRODUCTION

Sacbrood Virus (SBV) primarily affects the brood of the honeybee and results in larval death. Suitable detection methods are needed to control and eradicate SBV. Several detection methods have been developed for identifying SBV such as immunodiffusion assays, radioimmunoassay and Enzyme Linked Immunosorbent Assay (ELISA) (Grabensteiner *et al*., 2001).

Classical diagnostic methods have been based on Electron Microscopy (EM) and Agar Gel Immunodiffusion (AGID) but since the development of PCR, several RT-PCR-based methods have been described for the detection of viruses (Kukielka and Sanchez-Vizcaino, 2009; Ke *et al*., 2006). The aim of the current study was to develop a novel method for the detection of SBV in a simple, rapid and effective manner.

MATERIALS AND METHODS

A set of specific primers was designed by targeting the sequence of SBV sequence (GenBank accession No. AF469603) using Primer 5.0. The nucleotide sequences of the primers are shown in Table 1.

Total RNA was extracted from SBV isolates using an RNA extraction kit (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's protocol. The RT-PCR reaction was carried out in a 25 μL reaction mixture containing 5×buffer 5 μL, 2.5 mM dNTPs 2 μL, 40 U μL^{-1} AMV Reverse Transcriptase 0.5 μL, 10 U μL^{-1} RNase Inhibitor 1 and 2 μL of template RNA.

RT-PCR conditions were as follows: RT at 50°C for 20 min and initial denaturation at 94°C for 30 sec and 30 cycles of PCR with 44°C for 30 sec and 55°C for 30 sec and extension at 72°C for 50 sec. The RT-PCR assay successfully amplified the target sequence of the SBV gene as observed by agarose gel electrophoresis.

Table 1: The reverse transcription Polymerase Chain Reaction (RT-PCR) primer sets

Primers	Sequence (5'-3')	pos	Length (bp)
CSB3F	TCT GTC AAC TCA CGC TAA	857-874	437
CSB4R	ACG ACA TAC CCG CAA A	1278-1293	-

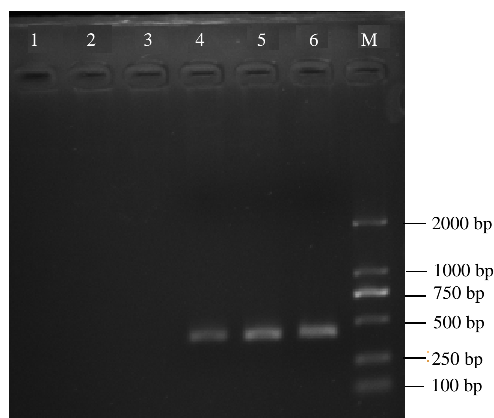

Fig. 1: Determination of the RT-PCR products by electrophoresed; Lane M: DL-2000 DNA marker; Lanes 1-3: PCR carried out for Deformed Wing Virus (DWV), Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV), respectively; Lane 4: +, positive control; Lane 5-6: SBV. All products were electrophoresed on 2% agarose gels and stained with ethidium bromide

Imaging was completed with 20 g L^{-1} agarose-TBE gel electrophoresis; DL-2000 Marker was regarded as the reference object of PCR products (Drolet *et al*., 2011). The result is shown in Fig. 1.

RESULTS AND DISCUSSION

The specificity of the RT-PCR assay was determined with SBV isolates and other honeybee viruses (Deformed Wing Virus (DWV), Chronic Bee Paralysis Virus (CBPV)

Corresponding Author: Jin-Long Yang, Chongqing Academy of Animal Science, 402460 Chongqing, China

and Kashmir Bee Virus (KBV). All SBV strains were positive whereas all other honeybee viruses were negative. This demonstrated that the RT-PCR assay was specific with no cross-reaction with the other honeybee viruses (Fig. 1). To evaluate the application of RT-PCR to detect SBV in clinical samples, the test was performed on 30 field clinical samples that were obtained from brood of the honeybee suspected of infection with SBV which yielded 22 positive and 8 negative by RT-PCR assay.

CONCLUSION

The current study presented the rapid detection of SBV by RT-PCR with analytic specificity. The assay is feasible for use in laboratories.

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REFERENCES

Drolet, B.S., H.M. Weingartl, J. Jiang, J. Neufeld and P. Marszal *et al*., 2011. Development and evaluation of one-step rRT-PCR and immunohistochemical methods for detection of Rift Valley fever virus in biosafety level 2 diagnostic laboratories. J. Virol. Methods, 10.1016/j.jviromet.2011.11.025.

Grabensteiner, E., W. Ritter, M.J. Carter, S. Davison and H. Pechhacker *et al*., 2001. Sacbrood virus of the honeybee (*Apis mellifera*): Rapid identification and phylogenetic analysis using reverse transcription-PCR. Clin. Diagn. Lab. Immunol., 8: 93-104.

Ke, G.M., H.L. Cheng, L.Y. Ke, W.T. Ji and J.L.C. Chulu *et al*., 2006. Development of a quantitative Light Cycler real-time RT-PCR for detection of avian reovirus. J. Virol. Methods, 133: 6-13.

Kukielka, D. and J.M. Sanchez-Vizcaino, 2009. One-step real-time quantitative PCR assays for the detection and field study of Sacbrood honeybee and Acute bee paralysis viruse s. J. Virol. Methods, 161: 240-246.