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P-048

Prevention of Korean Sacbrood Virus Infection in *Apis cerana* using dsRNA

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Introduction: Sacbrood virus (SBV), a causative pathogen of larval death in honeybees, is one of the most devastating diseases in bee industry throughout the world. Since 2010, the Korean Sacbrood virus (KSBV) caused great losses in Korean honeybee (Apis cerana) colonies. However, there is no treatment method culture for honeybee viruses including SBV. RNA interference (RNAi) is a gene-silencing technology by which small double-stranded RNAs are used to target the degradation of RNA with complementary sequence.

Materials and Methods: In this study, we report on prevention of SBV infection by feeding with double-stranded RNA. SBV sequences corresponding to a segment of structural protein VP1 gene for dsVP1 and a segment of the structural polyprotein open reading frame for dsSBV1 were used for cloning. dsRNA synthesis was carried out according to the protocol of the mMESSAGE mMACHINE T7 kit, it was treated with food. The feeding activity and mortality of larvae were observed every day. Experiments were carried out to examine whether ingestion of dsRNAs of SBV sequences would protect bees from SBV infection.

Results: The result indicated that two SBV-derived dsRNAs (dsSBV1 and dsVP1) protected bee larvae from subsequent SBV infection.

Conclusions: dsRNA of SBV will be used as an efficient and feasible way of controlling bee viral disease aw well as SBV.

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P-049

Development of Multiplex PCR for the Rapid Detection of Six Honeybee Viral Disease

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Introduction: Virus infections of the honeybee(Apis mellifera) have been increasingly investigated during the last decade. In general, honeybee viruses are widespread and most of them persist as inapparent infections. We screened honeybee colonies for the presence of several bee viruses, including sacbrood virus(SBV), black queen cell virus(BQCV), Kashmir bee virus(KBV), deformed wing virus(DWV), Israeli acute paralysis virus(IAPV) and acute bee paralysis virus (ABPV), using uniplex RT-PCR. Frequently simultaneous infections with different viruses are diagnosed in seemingly healthy bee colonies. Therefore we developed a multiplex RT-PCR assay for the simultaneous detection of multiple bee viruses.

Materials and Methods: Bee samples used for virus screening were collected from all over the country. Viral RNA was extracted using viral gene-spin viral DNA/RNA extraction kit(Intron). Specific primer for SBV, BQCV, KBV, DWV, IAPV and ABPV were designed based on the published nucleotide sequences. To screening for virus infection in honeybee colonies, brood were tested for the presence of honeybee viruses separately by the uniplex RT-PCR. uniplex PCR assay was performed using Top simple DryMix PCR kit (Enzynomics). Multiplex PCR assay was performed using 2X Master/MultiMAX PCR Kit (Intron). The PCR Products were electrophoresed in 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. It was cloned in Escherichia coli pGEM-T Easy vector(Promega).

Results: The use of broad-range primers enable us to detect a maximum of viral variants ideal to declare honeybee colonies virus. The SBV, BQCV, KBV, DWV, IAPV and ABPV primer sets, when used together in the multiplex reaction, amplified only specific products of the expected sizes of 192, 317, 413, 479 ,725, 900bp, respectively, which could be easily distinguished by agarose gel electrophoresis. And viral clones were used as positive controls in field sample tests. And sequences of each band were confirmed.

Conclusions: The main objective of this study was to develop a tool that detects the variants of bee viral species infecting honeybee. We expect to apply of a multiplex RT-PCR assay for offers a significant time and cost-saving advantage, especially when a lot of samples are analyzed.

P-050

Prevalence Survey for Severe Fever with Thrombocytopenia Syndrom Virus in Hard Ticks in Jeju, 2013-2014

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Introduction: Severe fever with thrombocytopenia syndrome