Virology 432 (2012) 155–161

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Analysis of the complete genome sequence of two Korean sacbrood viruses in the Honey bee, Apis mellifera

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ARTICLE INFO

Article history:
Received 13 March 2012
Returned to author for revisions
29 May 2012
Accepted 1 June 2012
Available online 1 July 2012

Keywords:
Apis mellifera
Complete genome sequence
Honey bee
Iflavirus
Sacbrood virus

ABSTRACT

The complete genomic RNAs of two Korean sacbrood virus (SBV) strains, which infect the honey bee, *Apis mellifera*, were sequenced. The two sequences (AmSBV-Kor19, AmSBV-Kor21) were distinguished by the presence or absence of a *Pst*I restriction site. These strains comprised of 8784 bp and 8835 bp; contained a single large ORF (179-8707 and 179-8758) encoding 2843 and 2860 amino acids, respectively. Deduced amino acid sequences comparison with some insect viruses showed that regions of helicase, protease and RdRp domains; structural genes were located at the 5′ end and non-structural genes at the 3′ end. Multiple sequence alignment showed that AmSBV-Kor19 was missing a section between nucleotides 2311 and 2361 (present in SBV-UK and CSBV) but was similar to that of the Korean SBV strain that infects *A. cerana* (AcSBV-Kor). The differences in the AmSBV-Kor19 strain may be the result of the virus adapting to a different host.

Introduction

Sacbrood virus (SBV) is a common virus that infects the honey bee (*Apis mellifera*). SBV has been detected in almost all the colonies throughout the world (Allen and Ball, 1996; Ellis and Munn, 2005). SBV infects mainly larvae but also adult bees, although they present no disease symptoms (Berenyi et al., 2006), and causes the death of larvae. Sacbrood virus was first described in 1913, but SBV was not characterized until 1964 (Bailey et al., 1964). SBV is one of many insect viruses generally referred to as picornavirus-like (Moore et al., 1985). Recently, it was reclassified into the genus *Iflavirus*, which contains linear positive single-stranded RNA viruses (Mayo, 2002; Baker and Schroeder, 2008). Complete genome sequences of many viruses are now available on GenBank. The genomes of these viruses are organized in three different ways: (i) monopartite and bicistronic, with replicase proteins encoded at the 5′ end and capsid proteins encoded at the 3′ end (Sasaki and Nakashima, 1999); (ii) monopartite and bicistronic, with replicase proteins encoded at the 5′ end and capsid proteins encoded at the 3′ end, however, the two ORFs overlap slightly (van der Wilk et al., 1997); (iii) monopartite and monocistronic, with capsid proteins encoded at the 5′ end and replicase proteins encoded at the 3′ end (Ghosh et al., 1999).

The complete genomic sequence of SBV-UK (GenBank accession number: AF092924) was first determined by Ghosh et al. (1999). SBV is a round (28 nm in diameter), non-enveloped virus with an 8832 bp RNA sequence encoding a polyprotein of 2858 amino acids (Ghosh et al., 1999). The SBV genome is monopartite monocistronic, with the structural genes arranged at the 5′ end and the non-structural genes at the 3′ end (Ghosh et al., 1999; Grabensteiner et al., 2001; Zhang et al., 2001; Chen et al., 2006; Ma et al., in press). The genome contains one large open reading frame (ORF) starting at nucleotide 179 and ending with a UAG stop codon at nucleotide 8775.

Chinese sacbrood virus (CSBV) sequences were identified by Zhang et al. (2001) and Ma et al. (in press) (GenBank accession numbers: AF469603 for CSBV-GZ and HM237361 for CSBV-LN). CSBV is similar to SBV-UK in terms of its physiological and biochemical features, but the viruses differ in their antigenicity and do not show cross infection. Sequence analysis indicates that

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http://dx.doi.org/10.1016/j.virol.2012.06.008

CSBV is different, but highly homologous to SBV-UK (Zhang et al., 2001). The CSBV genome comprises positive single-stranded RNA, encoding four structural proteins. The genomes of CSBV-GZ and CSBV-LN comprise 8740 bp and 8863 bp with a single large ORF encoding polyproteins of 2861 amino acids and 2884 amino acids, respectively (Zhang et al., 2001; Ma et al., in press).

In Korea, SBV was first detected in 2008, and then developed and broken out epidemic, especially serious in 2010. The infection with SBV has been detected in 75% colonies in Korea, during the year 2010 (the data unpublished). The sacbrood virus infection of the Korean honey bee, *Apis cerana* (AcSBV-Kor), was identified and its 8792 bp genome sequence published in GenBank (GenBank accession number: HQ322114) (Lee et al., 2010). Nucleotide alignment of partial sequences from the structural genes in the AcSBV-Kor genome (nucleotide sequences published in GenBank under accession numbers HQ916827–HQ916837) showed 93.9% and 93.7% similarity to the SBV-UK and CSBV-GZ strains, respectively (Choe et al., 2011). Previous studies detected AmSBV-Kor in the honey bee, *Apis mellifera* (Kim et al., 2008; Kim Cuc et al., 2008, 2009; Choi et al., 2010; Yoo et al., 2012), but none have analyzed its complete genome sequence. Moreover, the AmSBV-Kor strains were distinguished by the presence or absence of a *Pst*I restriction site. The position of *Pst*I has been detected only in CSBV but in SBV-UK (Le Quang Trung et al., 2010). Therefore, the aim of the present study was to determine the complete nucleotide sequence of SBV in the Korean honey bee, *Apis mellifera*, and compare it with some of other insect viruses published in GenBank.

Results

RT-PCR/RFLP analysis

All of the samples were first tested using three primer pairs to confirm SBV-positive infection (Table 1). RT-PCR was performed with specific primer sets (Le Quang Trung et al., 2010) to amplify the SBV genome region between nucleotides 5877 and 6477 according to reference strain AF092924. These SBV nucleotide sequences were submitted to the GenBank database under accession numbers JQ267669–JQ267675. The PCR products were digested with *Pst*I at 37 °C for 2 h (Le Quang Trung et al., 2010), electrophoresed in a 2% agarose gel, and stained with ethidium bromide. The results showed that three of the seven samples (Kor17, Kor19, and Kor20) were digested into two fragments of 411 bp and 186 bp (Figs. 1 and 2). Samples (Kor19 containing the *Pst*I restriction site and Kor21 without) were used to determine the full genome nucleotide sequence of AmSBV-Kor.

Nucleotide sequence analysis

The complete genome nucleotide sequences of these two Korean AmSBV strains were determined and deposited in GenBank under accession numbers: JQ390592 for AmSBV-Kor19 (*Pst*I restriction site) and JQ390591 AmSBV-Kor21 (no *Pst*I restriction site) Table 2.

The nucleotide sequences of the AmSBV-Kor19 and AmSBV-Kor21 genomes comprised 8784 bp and 8835 bp (excluding the poly A tail), respectively. The base composition of AmSBV-Kor19 was A(29.59%), C(16.41%), G(24.45%), and U(29.53%), and that of AmSBV-Kor21 was A(29.70%), C(16.11%), G(24.65%), and U(29.53%). The AmSBV-Kor19 genome contained a single large ORF encoding 2843 amino acids, commencing at nucleotide 179 and ending with a stop codon at nucleotide 8707. The AmSBV-Kor21 genome contained a single large ORF encoding 2860 amino acids, commencing at nucleotide 179 and ending with a stop codon at nucleotide 8758. Multiple sequence comparisons showed that the 5′ sequence of both strains was similar to that of the SBV-UK strain. AmSBV-Kor19 showed a closer genetic relationship to CSBV-GZ and CSBV-LN (94% and 92%, respectively) than to SBV-UK (90%), but was closest to AcSBV-Kor (up to 97%). However, AmSBV-Kor21 showed a closer genetic relationship to SBV-UK (93%) than CSBV-GZ, CSBV-LN or AcSBV-Kor (90%) (Table 3).

Multiple sequence alignment also showed that the sequence of AmSBV-Kor19 was missing a section between nucleotides 2311 and 2361 (present in SBV-UK and CSBV) and was identical to that of AcSBV-Kor. When compared with the CSBV-GZ strain, a consecutive 51-nucleotide deletion was identified within the polyprotein-coding region. Similar comparisons with SBV-UK and CSBV-LN identified a 48-nucleotide deletion and a 12-nucleotide deletion, respectively. The sequence of AmSBV-Kor21 did not show any nucleotide deletions in this section of the genome (Fig. 3). Thus, AmSBV-Kor21 was three nucleotides longer than SBV-UK, and 39 nucleotides longer than CSBV-LN.

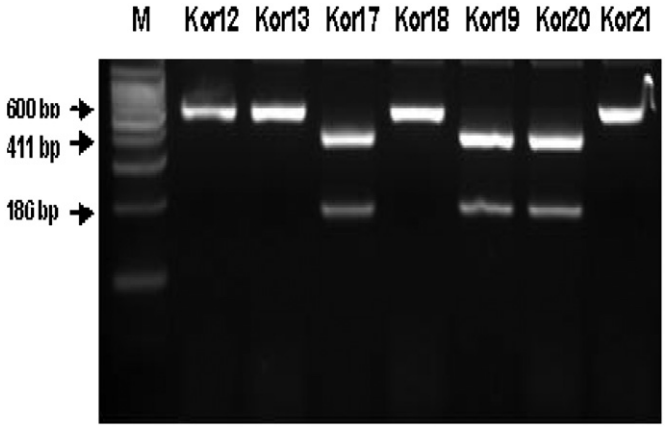

Fig. 1. PCR products were digested with *Pst*I and electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Lane M, DNA size markers (100 bp ladder); lanes 2–7: SBV samples from *A. mellifera*.

Table 1
Primer sets used to detect SBV and to amplify the region containing the *Pst*I restriction site.

Primer	Sequence 5′–3′	Length (bp)	Reference
CSBV	F: 5′-GGA TGA AAG GAA ATT ACC AG-3′ R: 5′-CCA CTA GGT GAT CCA CAC T-3′	426	Tentcheva et al. (2004)
SBV	F: 5′-ACC AAC CGA TTC CTC AGT AG-3′ R: 5′-CCT TGG AAC TCT GCT GTG TA-3′	487	Grabensteiner et al. (2001)
SBVR2	F: 5′-ACC AAC CGA TTC CTC AGT AG-3′ R: 5′-TCT TCG TCC ACT CTC TCA TCA C-3′	258	Yoo and Yoon (2009)
SBV-VN	F: 5′-AGGGAAATTACTAATATACCTTGCC-3′ R: 5′-TGGGTGTTCGTTCACTTGTTGAAAG-3′	600	Le Quang Trung et al. (2010)

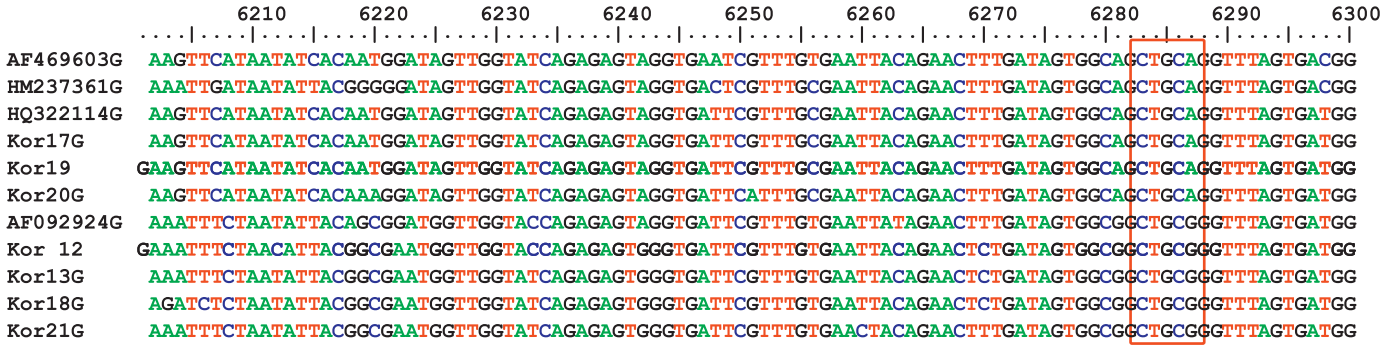

Fig. 2. Position of the *Pst*I restriction site in the sequences of the AmSBV-Kor strains.

Table 2
Primers used to obtain the complete nucleotide sequence of Korean SBV isolated from A. mellifera.

Primer	Sequence (5′–3′)	Position	Size of amplicons (bp)
SBV_F1	TACGAATCGTGATTCGATTC	1–20	678
SBV_R1	CAGGGGGACGCTACACAGCA	659–678	
SBV_F2	AGCTGCTAAGAGTATATTGG	579–598	770
SBV_R2	GTCCCATTGACCCAGATGGA	1330–1349	
SBV_F3	AATGATATGTTTATACGACC	1274–1293	753
SBV_R3	GGCTAGCGCCTATTTACCGG	2008–2027	
SBV_F4	TCAGTACATTTTACTGTGCC	1955–1974	690
SBV_R4	GCCGCCTTCTAGAATGATGC	2626–2645	
SBV_F5	TTTTGCGTAGACCAGTGTTG	2550–2569	729
SBV_R5	CCAGAGGGTTTTAGTTTGAA	3251–3270	
SBV_F6	AAGTTCAGATGGATGATAG	3177–3195	693
SBV_R6	ATATCACCGTTGTCTGGAGG	3851–3870	
SBV_F7	CCACGCCCAGTTGTGCAGGC	3761–3780	698
SBV_R7	GGTCTGGTTATAGGGATCAA	4440–4459	
SBV_F8	GGAGTTAATTTAAAACGACC	4376–4395	689
SBV_R8	CGACTGGGTTTCTTCCTAGT	5046–5065	
SBV_F9	GGATCTTTGCGTTTGGAAGA	4967–4986	698
SBV_R9	CGATGAGTGAGAGAACAGCG	5646–5665	
SBV_F10	AGTTGATAAGGGAGTTAATG	5548–5567	722
SBV_R10	AGAACTTTGATAGTGGCGGC	6251–6270	
SBV_F11	CAGCCTCACTGGATGAGAGC	6149–6168	715
SBV_R11	CGTAATCCAGTGCTTAAGGA	6845–6864	
SBV_F12	GAATGTTTAAGGATATAAGG	6750–6769	723
SBV_R12	TCGAAAATGAAACCCCTGGT	7454–7473	
SBV_F13	GTGTAAGAAACATGGAAGGC	7369–7388	694
SBV_R13	GCGGTGGACTATGGAGCATG	8044–8063	
SBV_F14	GATTATTCAAATTTTGGTCC	7979–7998	712
SBV_R14	GCATTAATTAAGCGGAAAAT	8672–8691	
SBV_F15	AGTAACAGATAAATATAAGG	8329–8348	503
SBV_R15	GGATTAATATCGATATATGGCATTTT	8807–8832	
SBV_F16	ATTAAGCGGAAAATACAACC	8678–8697	

Reference: CSBV-GZ (GenBank accession no. AF469603) and SBV-UK (GenBank accession no. AF092924) strains.

Table 3
Nucleotide sequence homology (%) between AmSBV-Kor and the other reference sequences.

	SBV-UK AF092924	CSBV-GZ AF460903	CSBV-LN HM237361	AcSBV-Kor HQ322114
AmSBV-Kor19	90%	94%	92%	97%
AmSBV-Kor21	93%	90%	90%	90%

Homology (%) of the deduced amino acid sequences for the coding regions between AmSBV-Kor and the other reference sequences

	SBV-UK AF092924	CSBV-GZ AF460903	CSBV-LN HM237361	AcSBV-Kor HQ322114
AmSBV-Kor19	94.7%	95.2%	92.8%	96.5%
AmSBV-Kor21	97.7%	94.7%	93.8%	94.9%

Amino acid sequence analysis

The deduced amino acid sequences for AmSBV-Kor and the other virus strains were then aligned and compared. The results showed the structural proteins in the 5′ end and the nonstructural proteins in the 3′ end. The helicase domains A, B, and C (Koonin and Dolja, 1993) with highly conserved amino acids within the first two domains, GxxGxGKS and Qx5DD, were found between

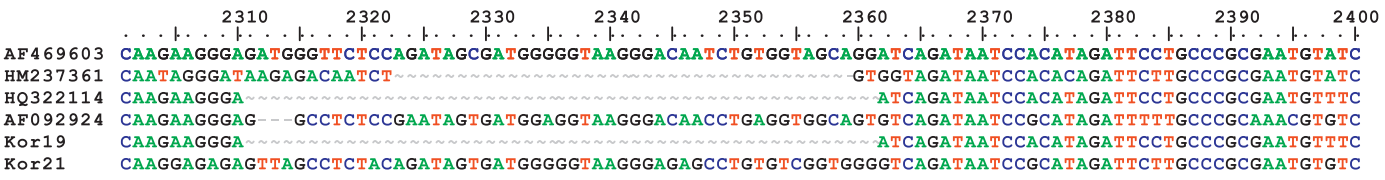

Fig. 3. Alignment of the nucleotide sequences of AF469603 (CSBV-GZ), HM237361 (CSBV-LN), HQ322114 (AcSBV-Kor), and AF092924 (SBV-UK) with those of AmSBV-Kor (Kor19 and Kor21) across the region between nucleotides 2311 and 2361. In comparison with CSBV-GZ (AF469603), CSBV-LN (HM237361), AcSBV-Kor (HQ322114), and SBV-UK (AF092924), AmSBV-Kor19 was missing a section between nucleotides 2311 and 2361 that was identical to AcSBV-Kor but was similar to AmSBV-Kor21.

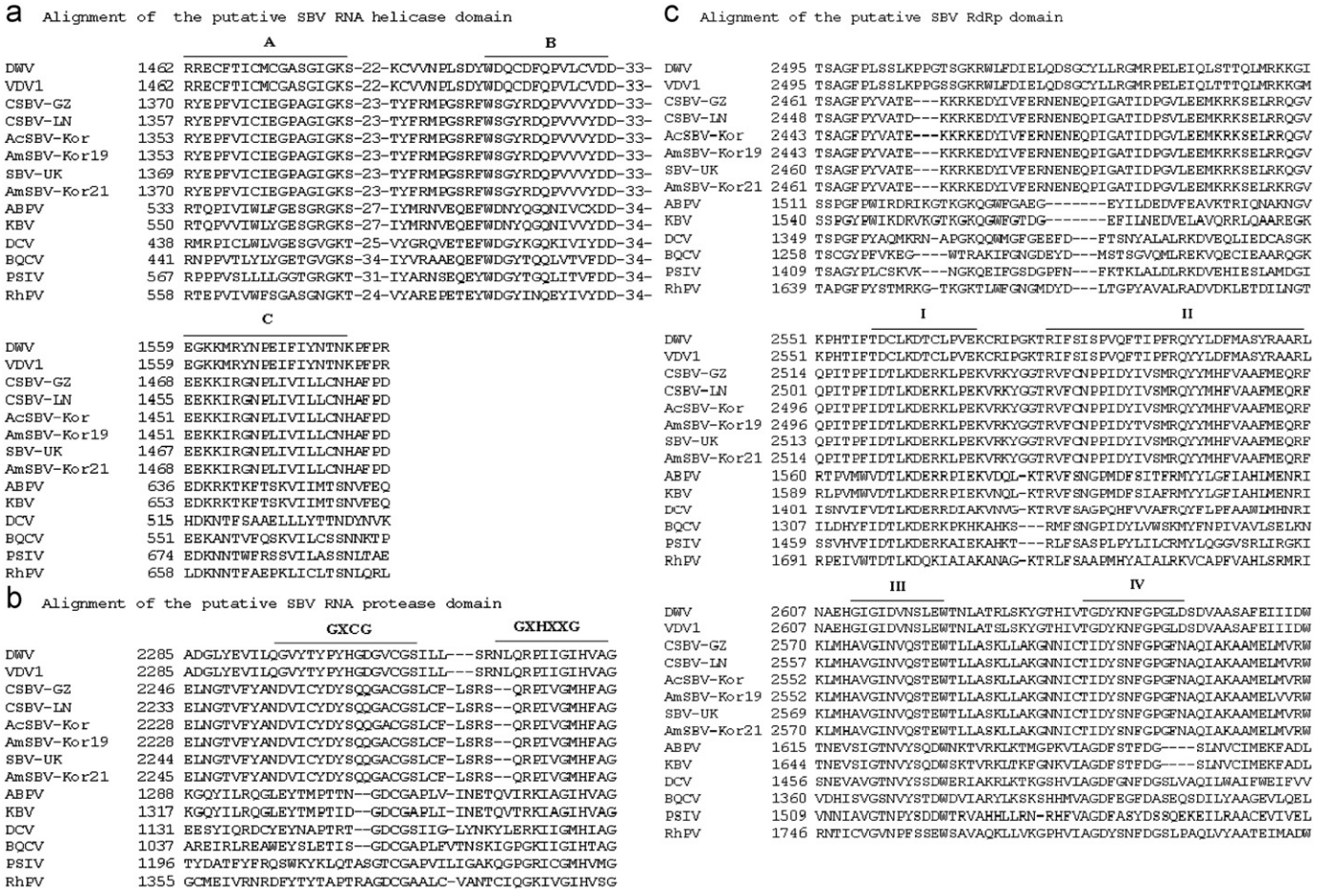

Fig. 4. Alignment of putative RNA helicase, protease and RdRp domains from AmSBV-Kor, AcSBV-Kor, CSBV, SBV-UK, ABPV, BQCV, DCV, DWV, KBV, PSIV, RhPV, and VDV1. The motifs identified for helicase are labeled A, B, C (Koonin and Dolja, 1993); the GxCG and GxHxxG motifs are identified for protease (Ongus et al., 2004); the motifs identified for RdRp are labeled I–VIII (Koonin and Dolja, 1993).

amino acids 1353 and 1489 in SBV strains (Fig. 4). The similar result showed in the *Iflaviridae* family including of Deformed wing virus (DWV, AY292384) and *Varroa destructor* virus (VDV1, AY251269) at the amino acid position between 1462 and 1580. These positions were found between amino acids 438 and 695 in the *Dicistroviridae* family including Acute bee paralysis virus (ABPV, AF150629), Black queen cell virus (BQCV, AF183905), *Drosophila* C virus (DCV, AF014388), Kashmir bee virus (KBV, AY275710), Plautia stali intestine virus (PSIV, AB006531) and *Rhopalosiphum padi* virus (RhPV, AF022937).

The equivalent of the GxCG and GxHxxG domains were identified within the protease domain in deduced amino acid sequences of viruses. These motifs were found between amino acids 2228 and 2328 in the *Iflaviridae* family, and from amino acid position 1037 to 1398 in the *Dicistroviridae* family. Eight conserved domains were identified in RdRp amino acid sequences between amino acids 2443 and 2835 in the *Iflaviridae* family, and from amino acid position 1258 to 1994 in the *Dicistroviridae* family.

Analysis of the structural protein regions indicated that the AmSBV-Kor19 and AcSBV-Kor had a 17-amino acid deletion at amino acid positions 710–728 in comparison with CSBV-GZ and AmSBV-Kor21. When compared with the SBV-UK and CSBV-LN, the deleted region was 17-amino acids and 4-amino acids, respectively (Fig. 5).

Next, we determined the homology of the deduced amino acid sequences for the polyprotein-coding region between AmSBV-Kor and the other strains. The results (Table 3) showed that AmSBV-Kor19 was 95.2% and 92.8% homologous to CSBV-GZ and CSBV-LN, respectively; 94.7% homologous to SBV-UK; and 96.5% homologous to AcSBV-Kor at the amino acid level. AmSBV-Kor21 was 94.7%, 93.8%, and 94.7% homologous to CSBV-GZ, CSBV-LN, and AcSBV-Kor at the amino acid level, respectively, and 97.7% homologous to SBV-UK.

```
CSBV-GZ      702 KPSNRPRREMGSPDSDGGKGQSVVAGSDNPHRFLPANVSNRWNEYSSAYL
CSBV-LN          KPTN----------SNRDKRQSVVD---NPHRFLPANVSNRWNEYSSVYL
AcSBV-Kor        KPPNRPRRE----------------SDNPHRFLPANVSNSWNEYSSAYL
AmSBV-Kor19      KPPNRPRRE----------------SDNPHRFLPANVSNSWNEYSSAYL
SBV-UK           KPPNRSRRE-ASPNSDGGKGQPEVAVSDNPHRFLPANVSNRWNEYSSAYL
AmSBV-Kor21      KPPNRSRRELASTDSDGGKGEPVSVGSDNPHRFLPANVSNRWNEYSSAYL
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Fig 5. In comparison with CSBV-GZ and AmSBV-Kor21, the AmSBV-Kor19 and AcSBV-Kor had a 17-amino acid deletion at amino acid positions 710-728 in comparison with CSBVGZ and AmSBV-Kor21. When compared with the SBV-UK and CSBV-LN, the deleted region were 17-amino acids and 4-amino acids, respectively.

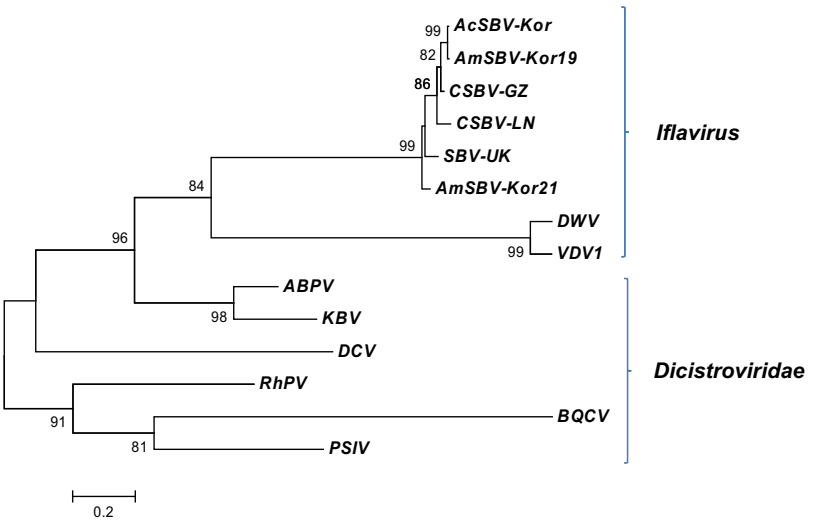

Fig. 6. Phylogenetic analysis of the RdRp domains. Tree was contructed from RdRp sequences of AcSBV-Kor, AmSBV-Kor19, AmSBV-Kor21, CSBV-GZ, CSBV-LN, SBV-UK, ABPV, BQCV, DCV, DWV, KBV, PSIV, RhPV and VDV1.

Phylogenetic analysis

A phylogenetic tree was constructed based on the RdRp sequences to illustrate the probable genetic relationships between the virus strains (Fig. 6). The RdRp tree segregated the viruses into two groups according to their taxonomic classification (*Iflavirus* and *Dicistroviridae*). In the *Iflavirus* group, DWV and VDV1 were clustered together such as a subgroup and SBV strains were clustered together in other subgroup. In the *Dicistroviridae* group, ABPV and KBV seemed to have a closely relationship. Whereas BQCV, PSIV, and RhPV tended to group together and DCV was intermediate branch.

The phylogenetic tree also showed that AmSBV-Kor21 was classified into the same branch as the SBV-UK strain, whereas the AmSBV-Kor19 strain was classified into a branch containing the CSBV and AcSBV-Kor strains. These results indicate that the AmSBV-Kor19 strain shows a very close genetic relationship with the CSBV strains, specifically the AcSBV-Kor strain.

Discussion

In the present study, the nucleotide sequences of two Korean SBVs infecting *A. mellifera* were determined. The AmSBV-Kor genome were monopartite monocistronic and contained a single large ORF starting at nucleotide 179 and terminating in a stop codon at nucleotide 8707 for AmSBV-Kor19 and 8758 for AmSBV-Kor21, respectively. The genomic organization of AmSBV-Kor clearly resembles that of *Iflavirus* family with structural proteins at the 5′ end and the nonstructural proteins at the 3′ end arranged in a similar order. Analysis of deduced amino acid sequences showed that AmSBV-Kor consisted of the conserved motifs within the helicase, protease, and RdRp domains as same as the other viruses.

Both contained a base composition very similar to that of other SBV strains, including SBV-UK, CSBV-GZ, and CSBV-LN (Ghosh et al., 1999; Zhang et al., 2001; Ma et al., in press). The AmSBV-Kor21 strain was similar to the SBV-UK strain and was classified into the same group. However, the nucleotide sequence of the AmSBV-Kor21 strain was three nucleotides longer (2312–2314) than that of SBV-UK, resulting in a deduced amino acid sequence that was one amino acid longer.

Whereas, the AmSBV-Kor19 strain showed different characteristics when compared with both the SBV-UK and CSBV strains. AmSBV-Kor19 infecting *A. mellifera* contained a *Pst*I restriction site within its nucleotide sequence (as did the CSBV and AcSBV-Kor strains). The AmSBV-Kor19 strain also formed a closely related cluster with the CSBV-GZ and CSBV-LN strains, with nucleotide sequence identities of 94 and 92%, respectively. AmSBV-Kor19 showed the highest sequence homology (97%) with AcSBV-Kor. The nucleotide sequence of AmSBV-Kor19 showed a deletion between nucleotides 2311 and 2361, as did the AcSBV-Kor strain. The deduced amino acid sequence of the AmSBV-Kor19 strain was 17 amino acids shorter than that of SBV-UK and 18 amino acids shorter than that of the AmSBV-Kor21 strain.

This study identified two SBV strains infecting the Korean honey bee, *A. mellifera*, which were distinguished by the presence/absence of a *Pst*I restriction site. The AmSBV-Kor21 strain was very similar to the SBV-UK strain, whereas the AmSBV-Kor19 strain showed characteristics common to both the CSBV and SBV-UK strains. These results suggest that some of the differences in the AmSBV-Kor19 strain detected in this study may be the result of the virus adapting to a different host, confirming the conclusions made by Grabensteiner et al. (2001).

Materials and methods

Sample collection

A total of seven *A. mellifera* larvae samples were obtained from three provinces in Korea (Gyeonggi, Chungbuk, and Jeonbuk) in 2011.

RNA extraction and RT-PCR/RFLP analysis.

Collected samples (each sample including 20 larvae from a single colony) were completely homogenized in 3 ml sterile PBS to yield a 10% (w/v) solution using a pestle and mortar. The samples were centrifuged for 3 min at 16,000 × *g* and the supernatant was used for RNA extraction using an RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Total RNA was eluted in 30 μl of elution buffer and used directly for RT-PCR. Extracted RNA was used to synthesize cDNA using oligo (dT) 12–18 as the primer (Invitrogen, Carlsbad, CA, USA).

All of the samples were first tested with three primer pairs to confirm SBV-positive infection (Table 1). RT-PCR was carried out with specific primer sets (Le Quang Trung et al., 2010) under the following conditions: 50 °C for 30 min, 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min in a C1000 Thermal Cycler (Bio-Rad, USA). The RT-PCR products were digested by *Pst*I (TaKaRa, Japan) at 37 °C for 2 h, electrophoresed in a 2% agarose gel, stained with ethidium bromide, and photographed under UV light.

cDNA synthesis and amplification-specific fragments.

To amplify the internal region of the Korean SBV genome, 15 pairs of primers were designed based on the sequence of the Chinese (CSBV-GZ, GenBank accession No. AF469603) and UK strains (SBV-UK, GenBank accession No. AF092924). cDNAs were synthesized using each reverse primer and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). A 40 μl volume of RT-PCR reaction mixture, including 10 μl extracted RNA, was incubated at 50 °C for 1 h and the reaction was terminated at 75 °C for 10 min. 5 μl of each cDNA was PCR-amplified with Platinum® Taq DNA polymerase high fidelity (Invitrogen, Carlsbad, CA, USA) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min in a C1000 Thermal Cycler (Bio-Rad, USA).

The RACE kit (5′ RACE system for Rapid Amplification of cDNA Ends, Invitrogen, Carlsbad, CA, USA) was used to amplify the 5′ terminal region of the SBV genome, according to the manufacturer's instructions. The amplified products were purified by QIAquick Gel Extraction kit (Qiagen, Germany). After gel extraction, each amplicon was cloned into the TA vector (Enzynomics, Korea) and sequenced bi-directionally with both M13 forward and reverse primers using an automated sequencer (ABI Prism 3700 Genetic Analyzer). To sequence the 3′ terminal region, the same procedure used to sequence the internal region was used but with an oligo(dT) oligonucleotide containing an adapter sequence at its 5′ end as the reverse primer.

Nucleotide sequencing and analysis.

The nucleotide sequence of each fragment was assembled to build a continuous complete sequence using the DNASTAR program. Multiple nucleotide and deduced amino acid sequence alignments were performed using published SBV sequences (GenBank accession numbers AF092924, HM237361, HQ322114 and AF469603) as references and BioEdit version 7.0.9.0 (Hall, 1999). A phylogenetic tree was constructed using the MEGA 4.1 package (Tamura et al., 2007) and the neighbor-joining (NJ) method (Saitou and Nei, 1987), and computed with the Kimura 2 parameter method (Kimura, 1980). A boot-strap value of 1000 replicates was applied to yield a robust phylogeny. The virus genome sequences (with accession numbers) used in this study were Acute bee paralysis virus (ABPV, AF150629); Black queen cell virus (BQCV, AF183905); Drosophila C virus (DCV, AF014388); Deformed wing virus (DWV, AY292384); Kashmir bee virus (KBV, AY275710); Plautia stali intestine virus (PSIV, AB017037); Rhopalosiphum padi virus (RhPV, AF022937) and Varroa destructor virus (VDV1, AY251269) (Govan et al., 2000; Leat et al., 2000; Johnson, Christian (1998); Lanzi et al., 2006; deMiranda et al., 2004; Sasaki et al., 1998; Moon et al., 1998, Ongus et al., 2004).

Acknowledgments

This study was supported by the Animal, Plant and Fisheries Quarantine and Inspection Agency, Korean Ministry of Agriculture and Forestry, Republic of Korea.

References

Allen, M.F., Ball, B.V., 1996. The incidence and world distribution of honey bee viruses. Bee World 77, 141–162.
Bailey, L., Gibbs, A.J., Woods, R.D., 1964. Sacbrood virus of the larval honey bee (*Apis mellifera* Linnaeus). Virology 23, 425–429.
Baker, A.C., Schroeder, D., 2008. The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (order Picornavirales) infecting *Apis mellifera* L. populations. Virol. J. 5, 10.
Berenyi, O., Bakonyi, T., Derakhshifar, I., Koglberger, H., Nowotny, N., 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl. Environ. Microbiol. 72 (4), 2414–2420.
Chen, Y., Evans, J., Feldlaufer, M., 2006. Horizontal and vertical transmission of viruses in the honeybee *Apis mellifera*. J. Invertebr. Pathol. 92, 152–159.
Choe, S.E., Nguyen, T.T.D., Hyun, B.H., Noh, J.H., Lee, H.S., Lee, C.H., Kang, S.W., 2011. Genetic and phylogenetic analysis of South Korean sacbrood virus isolates from infected honey bees (*Apis cerana*). Vet. Microbiol., 2011. (accepted 8 December).
Choi, Y.S., Lee, M.Y., Hong, I.P., Kim, N.S., Kim, H.K., Lee, K.G., Lee, M.L., 2010. Occurrence of sacbrood virus in Korean apiaries from *Apis cerana* (Hymenoptera: Apidae). J. Apic. 25, 187–191.
deMiranda, J.R., Drebot, M., Tyler, S., Shen, M., Cameron, C.E., Stoltz, D.B., Camazine, S.M., 2004. Complete nucleotide sequence of Kashmir bee virus and comparison with Acute bee paralysis virus. J. Gen. Virol. 85 (8), 2236–2270.
Ellis, J.D., Munn, P.A., 2005. The worldwide health status of honey bees. Bee World 86, 88–101.
Ghosh, R.C., Ball, B.V., Willcocks, M.M., Carter, M.J., 1999. The nucleotide sequence of sacbrood virus of the honeybee: an insect picorna-like virus. J. Gen. Virol. 80, 1541–1549.
Govan, V.A., Leat, N., Allsopp, M., Davison, S., 2000. Analysis of the complete genome sequence of Acute bee paralysis virus shows that it belongs to the novel group of insect-infecting RNA viruses. Virology 277 (2), 457–463.
Grabensteiner, E., Ritter, W., Carter, M.J., Davision, S., Pechhacker, H., Kolodziezek, J., Boecking, O., Derakhshifar, I., Moosbeckhofer, R., Licek, E., Nowotny, N., 2001. Sacbrood virus of the honeybee (*Apis mellifera*): Rapid identification and phylogenetic analysis using reverse transcription-PCR. Clin. Diagn. Lab. Immunol. 8, 93–104.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alighment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Johnson, K.N., Christian, P.D., 1998. The novel genome organization of the insect picorna-like virus Drosophila C virus suggests this virus belongs to a previously undescribed virus family. J. Gen. Virol. 79 (1), 191–203.
Kim, H.K., Choi, Y.S., Lee, M.L., Lee, M.Y., Lee, K.G., Ahn, N.H., 2008. Detection of sacbrood virus (SBV) from the honeybee in Korea. Korean J. Apic. 23, 103–109.
Kim Cuc, N.T., Yoo, M.S., Kim, I.W., Kang, M.H., Han, S.H., Yoon, B.S., 2008. Development of PCR detection method for sacbrood virus in honeybee (*Apis mellifera* L.). Korean J. Apic. 23, 177–184.
Kim Cuc, N.T., Yoo, M.S., Kang, M.H., Han, S.H., Yun, C.H., Yoon, B.S., 2009. Development of Real-time PCR assay for the detection of sacbrood virus in honeybee (*Apis mellifera* L.). Korean J. Apic. 24, 15–21.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Koonin, E.V., Dolja, V.V., 1993. Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. Crit. Rev.Biochem. Mol, Biol.y 28, 375–430.
Lanzi, G., deMiranda, J.R., Boniotti, M.B., Cameron, C.E., Lavazza, A., Capucci, L., Camazine, S.M., Rossi, C., 2006. Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). J. Virol. 80 (10), 4998–5009.
Quang Trung Le, Dinh, Q.T., Tran, V.T., Truong, A.T., Tang, T.P., Dang, T.T., Phung, H.C., 2010. Application of multiplex RT-PCR/RFLP for early detection and genetic relationship research of sacbrood viruses on honeybee in Vietnam. J. Agric. Rural Dev. 1, 34–40.
Leat, N., Ball, B., Govan, V., Davison, S., 2000. Analysis of the complete genome sequence of black queen cell virus, a picorna-like virus of honey bees. J. Gen. Virol. 81 (8), 2111–2119.
Lee, C.H., Choe, S.E., Hyun, B.H., Kang, S.W., Jung, S.C., Song, J.Y., 2010. GenBank accession number HQ322114.
Ma, M., Li, M., Cheng, J., Yang, S., Wang, S., Li, P. Molecular and biological characterization of Chinese sacbrood virus LN isolate. Comparative Functional Genomics, in press.
Mayo, M.A., 2002. Virus taxonomy-Houston 2002. Arch. Virol. 147, 1071–1076.
Moon, J.S., Domier, L.L., McCoppin, N.K., D'Arcy, C.J., Jin, H., 1998. Nucleotide sequence analysis shows that Rhopalosiphum padi virus is a member of a novel group of insect-infecting RNA viruses. Virology 243 (1), 54–65.
Moore, N.F., Reavy, B., King, L.A., 1985. General characteristics, gene organization of human hepatitis A virus. Proc. Nat. Acad. Sci., USA 83, 8132–8136.
Ongus, J.R., Peters, D., Bonmatin, J.M., Bengsch, E., Vlak, J.M., VanOers, M.M., 2004. Complete sequence of a picorna-like virus of the genus *Iflavirus* replicating in the mite *Varroa destructor*. J. Gen. Virol. 85 (12), 3747–3755.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Sasaki, J., Nakashima, N., Saito, H., Noda, H., 1998. An insect picorna-like virus, Plautia stali intestine virus, has genes of capsid proteins in the 3′ part of the genome. Virology 244 (1), 50–58.
Sasaki, J., Nakashima, N., 1999. Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus in vitro. J. Virol. 73, 1219–1226.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M.E., Bergoin, M., 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera L.* and *Varroa destructor* mite populations in France. Appl. Environ. Microbiol. 70 (12), 7185–7191.
van der Wilk, F., Dullemans, A.M., Verbeek, M., van den Heuvel, J.F., 1997. Nucleotide sequence and genomic organization of *Acyrthosiphon pisum* virus. Virology 238, 353–362.
Yoo, M.S., Yoon, B.S., 2009. Incidence of honeybee disease in Korea 2009. Korean J. Apicu. 24(4), 273–278.
Yoo, M.S., Kim Cuc, N.T., Phan, V.N., Han, S.H., Kwon, S.H., Yoon, B.S., 2012. Rapid detection of sacbrood virus in honeybee using ultra rapid real-time polymerase chain reaction. J. Virol. Method 179, 195–200.
Zhang, J., Feng, J., Liang, Y., Cheng, D., Zhou, Z.H., Zhang, Q., Lu, X., 2001. Three-dimensional structure of the Chinese sacbrood bee virus. Sci. China 44, 443–449.