J Appl Biotechnol Rep. 2019 Mar;6(1):15-19

doi 10.29252/JABR.06.01.03

Journal of
Applied Biotechnology
Reports

Original Article

Highlights on the Genetic Relationships Between Some Honey Bee Viruses Using Various Techniques

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Received October 8, 2018; **Accepted** February 1, 2019; **Online Published** March 15, 2019

Abstract

Introduction: Honey bees are used intensively to boost the agricultural and economic sectors worldwide. Many viruses attack honey bees and cause severe problems to the bee colonies, and constitute a real challenge for beekeeping development. Hence, understanding the genetic characteristics of bee viruses is necessary to highlight the phylogenetic relationships between them, and to find out similarity aspects based on sequences.

Materials and Methods: Some public resources and free genetic analysis programs were utilized to perform this study. The complete sequences for some viruses were downloaded and analyzed using various programs and methods.

Results: Some viruses shared the same base composition pattern in regards to percentage of A, T, C, and G. The phylogenetic relationships among the investigated viruses were presented and discussed. The phylogenetic trees constructed using three bioinformatics programs based on different methods emphasized the relationship between Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV), and between deformed wing virus (DWV) and Kakugo virus (KV). These genetic relationships were also confirmed using enzymatic digestion to the sequences, gene cluster families, and open reading frames (ORFs).

Conclusions: This study has presented new trends to analyze genetic similarities between organisms utilizing sequences. Different results for the phylogenetic relationships could be obtained when performing the analysis using various methods without impacting the relationships between closely related organisms. This study encourages the performance of additional studies to figure out functional components of these viruses.

Keywords: Phylogeny, Honey Bees, Viruses, Bioinformatics

Citation: Abou-Shaara H. Highlights on the genetic relationships between some honey bee viruses using various techniques. J Appl Biotechnol Rep. 2019;6(1):15-19. doi:10.29252/JABR.06.01.03.

Introduction

Large numbers of honey bees, *Apis mellifera*, colonies are being kept worldwide to provide pollination services to plants, and to boost livelihood means at rural areas.[1-4] Hence, honey bees are the main target to many viral and non-viral diseases. Such diseases can greatly impact beekeeping negatively causing huge losses, and contribute in the colony collapse disorder, a phenomenon of sudden bee disappearance.[5,6] The viral diseases of honey bees can impact both immature and mature stages, for example, sacbrood virus (SBV), black queen cell virus (BQCV) for immature stages,[7,8] paralysis viruses and deformed wing virus (DWV) for mature stages.[9]

There are about 24 or more subspecies of *A. mellifera* and can be infected by many viral diseases. The bee viruses can be transmitted horizontally and vertically,[10] and also can be transmitted from bee species to another and from wild bees to domesticated ones.[11-15] Thus, these viruses are multi-host pathogens, including DWV and BQCV.[8] Also, similarities have been detected between some bee viruses and viruses infecting other insects, including acute bee paralysis virus.[16] Moreover, the ecto-parasites, *Varroa* mites can greatly cause the prevalence of some viruses between bee colonies.[17-21] Therefore, the spread of these viruses can happen via various means, and can cause severe damages to the colonies.

The recent development in bioinformatics provides new trends in analyzing genetic data. In fact, the genome sequences of many bee viruses are available and can be analyzed using the available bioinformatics programs. However, few studies have been conducted on honey bee viruses using bioinformatics to find out genetic similarities between them, and previous studies have mainly concentrated on the phylogenetic relationships.[22,23] Therefore, this study has aimed to highlight the genetic similarities between some common honey bee viruses, using various programs and methods. This can help expand the knowledge about these viruses, and help understand the co-infection of them to honey bees. Moreover, this study presents new information about their genetic characteristics which are poorly known.

Materials and Methods

Viruses

The complete genome sequences of some viruses were

obtained from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) and were included in the study (Table 1). The uracil (U) bases were changed to thymine (T) bases in these RNA viruses to facilitate the extraction of genetic characteristics utilizing the available data for other organisms.

Base Composition

The content of nucleotides (A, T, C, and G) in the sequences were analyzed and presented as percentages. This analysis shades more insights into the similarity aspects between these viruses based on their base composition.

The Phylogenetic Relationships

The phylogenetic relationships between these viruses were studied using three methods. First method; the sequences of the complete genome were aligned using Clustal alignment. Then, the phylogenetic tree was constructed based on the Percentage of Identity (PID) using Jalview 2.10.3.[24] Second method; Geneious tree builder using neighbor-joining method with global alignment with free end gaps as the alignment type was used to construct the phylogenetic tree by Geneious 1.13.1.[25] Third method; molecular phylogenetic analysis was done using maximum likelihood method based on the Jukes-Cantor model[26] and ClustalW to align the sequences using MEGA7.[27] The constructed trees from these methods were compared.

Digestion

BQCV JL1, DWV DJE202, IAPV, Kakugo virus (KV), KBV, and SBV strain II-9 (SBV) were used in this analysis and the next two analyses. The available enzymes at the Genome Compiler 2.2.88 (http://www.genomecompiler.com) were used to digest the genome of these 6 viruses. Then, ladder NEB 50 bp was used to show and compare the resulted fragments.

Clusters

The exact genes and their functions in viruses are not well studied. Therefore, the available databases with high similarity from other organisms to the sequences of these viruses were utilized. Thus, potential proteins were firstly downloaded from Uniprot (http://www.uniprot.org/), and then uploaded to OrthoVenn (http://www.bioinfogenome.net) to construct the shared clusters for the six viruses based on proteins.

Open Reading Frames

Firstly, open reading frames (ORFs) were detected in the sequences of the selected 6 viruses using start codons of ATG or GTG or TTG or CTG and minimum length of 60 amino acids considering all frame types. Secondly, only start codon of ATG for forward frames with length of 80 amino acids was used to detect specific ORFs. Genome Compiler was used to detect ORFs and to also compare the studied sequences.

Results and Discussion

Base Composition

Some viruses shared the same base composition pattern as shown in Table 2. Especially, strains of SBV had a high percentage of G followed by A, then T and finally C, while DWV, KV and BQCV had a high percentage of T, followed by A, then G and finally C. Kashmir bee virus (KBV) and all strains of Israeli acute paralysis viruses (IAPV) had a high percentage of A followed by T, then G, and finally C. The percentages of A ranged from 29.70% (SBV strain K3A) to 33.78% (KBV), T ranged from 28.86% (IAPV strain PP) to 32.26% (KV), C ranged from 15.89% (KV) to 18.58% (BQCV strain PP), and G from 20.20% (KBV) to 24.61% (SBV strain K3A). Variations between strains of the same virus were less than 1% in all bases, and this reflects the high genetic similarity between them. On the other hand, variations between viruses in their base composition can be explained by the variations in genetic characteristics, including genes and their functions of these viruses.

Phylogenetic Relationships

The phylogenetic tree (Figure 1) classified the viruses into

Table 1. Names, Accession Numbers and Number of Bases for the Included Honey Bee Viruses in the Study

Virus	Version	Accession No.	Base Number
Sacbrood virus strain II-9	JX270800.1	JX270800	8740
Sacbrood virus strain S2	JX270799.1	JX270799	8741
Sacbrood virus strain K3A	JX270798.1	JX270798	8756
Sacbrood virus strain K5B	JX270797.1	JX270797	8700
Sacbrood virus strain K1A	JX270796.1	JX270796	8743
Sacbrood virus strain II-2	JX270795.1	JX270795	8680
Deformed wing virus-DJE202	KJ437447.1	KJ437447	10167
Kakugo virus	AB070959.1	AB070959	10152
Kashmir bee virus	NC_004807.1	NC_004807	9524
Black queen cell virus isolate JL1	KP119603.1	KP119603	8358
Black queen cell virus strain PP	KY243932.1	KY243932	8511
Israeli acute paralysis virus strain Korea3	KC690270.1	KC690270	9470
Israeli acute paralysis virus strain Korea2	KC690269.1	KC690269	9480
Israeli acute paralysis virus strain Korea1	KC690268.1	KC690268	9478
Israeli acute paralysis virus strain PP	KY243933.1	KY243933	9500

Table 2. Base Composition of Studied Viruses

Virus	A%	T%	C%	G%
Sacbrood virus strain II-9	29.81	29.61	16.12	24.45
Sacbrood virus strain S2	29.80	29.14	16.62	24.42
Sacbrood virus strain K3A	29.70	29.23	16.44	24.61
Sacbrood virus strain K5B	29.81	29.51	16.16	24.50
Sacbrood virus strain K1A	29.90	29.62	16.02	24.44
Sacbrood virus strain II-2	29.75	29.52	16.31	24.40
Deformed wing virus-DJE202	29.34	31.86	16.22	22.55
Kakugo virus	29.41	32.26	15.89	22.43
Kashmir bee virus	33.78	28.55	17.45	20.20
Black queen cell virus isolate JL1	29.40	30.50	18.25	21.82
Black queen cell virus strain PP	29.50	30.13	18.58	21.77
Israeli acute paralysis virus strain Korea3	32.75	29.44	17.08	20.71
Israeli acute paralysis virus strain Korea2	32.94	29.32	17.06	20.66
Israeli acute paralysis virus strain Korea1	32.84	29.32	17.12	20.71
Israeli acute paralysis virus strain PP	32.52	28.86	17.69	20.91

groups based on their genome sequences. There are two main groups; one group is for KBV, IAPV and BQCV, while the other group is for SBV, DWV and KV. All strains of each virus were placed close to each other, suggesting the correctness of the tree. The phylogenetic tree based on Geneious tree builder classified the viruses into two major groups one for BQCV, KV and DWV and the other one for SBV, KBV and IAPV (Figure 2). The phylogenetic tree constructed based on maximum likelihood method showed the presence of two major groups; one group for SBV, KV and DWV and the other group for BQCV, KBV, and IAPV (Figure 3). The tree constructed by Geneious tree builder was different from the other trees in regards to the position of BQCV and SBV.

It is evident that the method used to construct the trees impacted the phylogenetic relationships between the viruses in the major groups (branches). This is while within the sub-groups (sub-branches) no variations were detected. Especially, in all the constructed trees KV and DWV were placed together in one branch, and all strains of SBV, BQCV, and IAPV were placed in separate branches. The constructed trees confirmed the relationship between KBV and IAPV as well as between KV and DWV using various methods. Accordingly, KBV and IAPV (Fam. Dicistroviridae) are closely related to each other.[28] Also, KV and DWV were placed close to each other within the same sub-group. The KV is suggested to be a sub-type of DWV, but KV showed a relationship with the

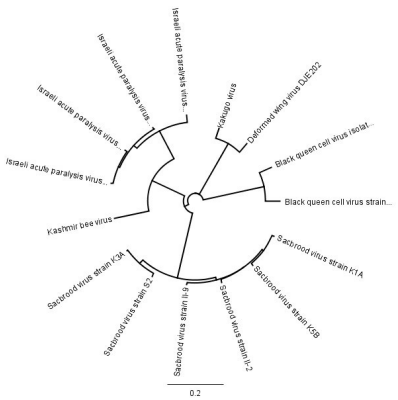

Figure 2. Phylogenetic Relationships Between Studied Viruses as Constructed Using Geneious Tree Builder.

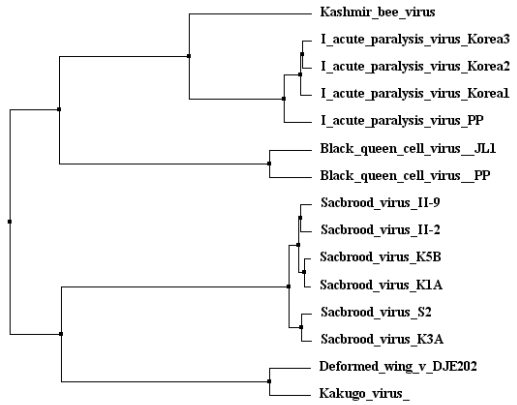

Figure 1. Phylogenetic Relationships Between Honey Bee Viruses Using Complete Genome Sequence Based on the Percentage of Identity Using Jalview.

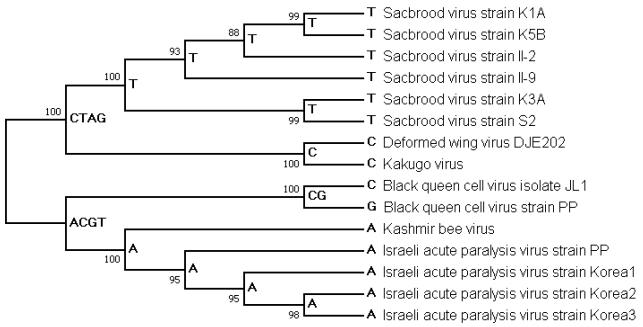

Figure 3. Phylogenetic Relationships Between Studied Viruses as Constructed Based on the Maximum Likelihood Method Using MEGA7. The letters (ACGT) denotes to the ancestors.

aggressive behaviour of honey bees.[29]

Digestion

The number of fragments ≥100 bp was 18, 21, 21, 23, 22, and 10 for QBCV, DWV, IAPV, KV, KBV, and SBV, respectively (Figure 4). The fragmentation pattern showed the presence of similarities between IAPV and KBV, and between KV and DWV. The fragments of QBCV were partially similar to DWV while few similarities were found between SBV and the other viruses. This is partially in line with the constructed phylogenetic trees in previous analysis.

Clusters

The Venn diagram shows the distribution of shared clusters among studied viruses. The number of shared clusters is 270 between IAPV and KBV, and 322 between DWV and SBV, 3 between IAPV and BQCV, and 2 between KBV and BQCV (Figure 5). These results confirm the trend obtained by the constructed trees, especially the relationships between IAPV and KBV, and between DWV and SBV.

Open Reading Frames

The detected ORFs using general search with 4 start codons for all type of frames and the specific search with one start codon ATG for only forward frames are shown in Figures 6 and 7, respectively. The presence of multi ORFs in the investigated sequences is evident from the figures. From the two figures, especially Figure 7, the pattern of the detected ORFs is similar between IAPV and KBV, and between KV and DWV. This supports the phylogenetic relationships highlighted between these viruses.

Conclusions

This study highlighted the genetic relationships between some honey bee viruses. Base composition was highly similar between strains of the same virus. Also, some viruses shared the same pattern of base percentages. Different results for the phylogenetic relationships could be obtained when performing the analysis using various methods without impacting the relationships between closely related viruses. Digesting the full sequences into fragments, identifying shared cluster families, and ORFs were used to support the genetic relationships between these viruses, and seem to be suitable trends. This study documented the close relationships between the KBV and the IAPV, and also between the DWV and the KV.

Figure 4. The Fragments of (A) Black Queen Cell Virus JL1, (B) Deformed Wing Virus, (C) Israeli Acute Paralysis PP, (D) Kakugo Virus, (E) Kashmir Bee Virus, and (F) Sacbrood Virus Strain II-9 After Digestion Using Enzymes Available at Genome Compiler. The first column is for ladder NEB.

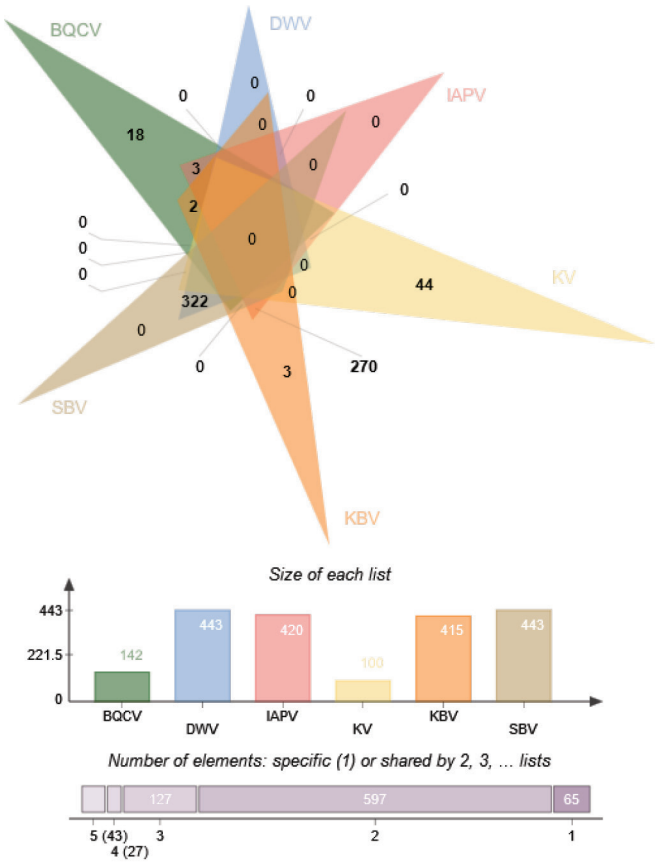

Figure 5. The Distribution of Shared Gene Families Among Studied Viruses. BQCV: Black queen cell virus JL1, DWV: Deformed wing virus, IAPV: Israeli acute paralysis PP, KV: Kakugo virus, KBV: Kashmir bee virus, and SBV: Sacbrood virus strain II-9.

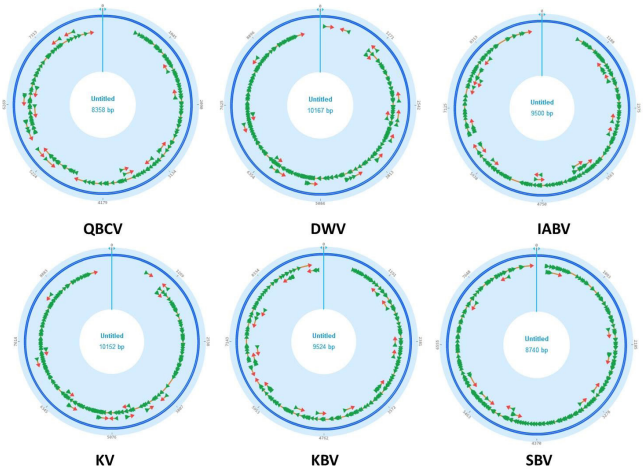

Figure 6. ORFs Detected for the Studied Viruses Using Start Codons of ATG or GTG or TTG or CTG Considering All Frame Types Using Genome Compiler. BQCV: Black queen cell virus JL1, DWV: Deformed wing virus, IAPV: Israeli acute paralysis PP, KV: Kakugo virus, KBV: Kashmir bee virus, and SBV: Sacbrood virus strain II-9.

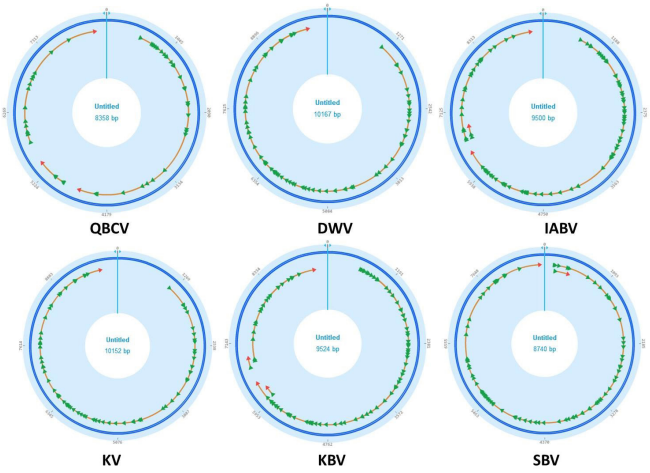

Figure 7. ORFs Detected for the Studied Viruses Using Start Codon of ATG for Forward Frames Using Genome Compiler. BQCV: Black queen cell virus JL1, DWV: Deformed wing virus, IAPV: Israeli acute paralysis PP, KV: Kakugo virus, KBV: Kashmir bee virus, and SBV: Sacbrood virus strain II-9.

Conflict of Interest Disclosures

The author declares he has no conflicts of interest.

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