



Comparison of Iflavirus Diversity in Agricultural and Forest Pest Lepidoptera

Gözde Büşra EROĞLU^{1*}

¹Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 25050, Erzurum/Turkey

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Abstract

Iflaviridae is a novel and important family of viruses in the *Picornavirales* order that infects invertebrates. Iflaviruses are non-enveloped, linear, positive single-stranded RNA viruses. The importance of iflaviruses in agriculture and forestry is twofold because these viruses, both cause economic loss by making insects such as honey bees and silkworms ill and prevent economic loss by making agricultural and forest pest insects ill. Therefore, iflaviruses contain highly interesting isolates that infect both beneficial and harmful insects in agriculture. Its genome structure, like most RNA viruses, has a very small genome size (8.8-10 kb), with a single open reading frame in the entire genome. The genomes of 14 infective iflaviruses that infect agricultural and forest pest insects from different countries have been analyzed so far. In this study, the similar relationship between these 14 viruses, whose complete genomes are available, was analyzed according to the complete sequence of the polyprotein. The results showed that the virus isolates obtained from forest pest insects were closely related to each other. Similarly, it was revealed that the iflaviruses obtained from insects that damage agricultural products are more similar to each other. In addition, the results from this study support previous studies on adding a new genus to the *Iflaviridae* family, which has only one genus.

Keywords: Iflaviruses, pest insects, phylogeny, Kimura-2 parameter

Introduction

The *Iflaviridae* is a new virus family established about 10 years ago (Carstens & Ball, 2009). It is in the *Picornavirales* order and consists of a single genus (*Iflavirus*). The family name is derived from the infectious flacherie virus, the type species of the genus

Iflavirus (1). Iflaviruses have spherical virions and a non-enveloped icosahedral structure with a diameter of approximately 22-30 nm (1-3). Members of the *Iflaviridae* family cause infection in many arthropods, especially in *Lepidoptera*, *Hymenoptera*, and *Hemiptera* orders (4). Therefore, iflaviruses are evaluated from two perspectives. The first is to protect beneficial insects from this viral disease by biologically controlling isolates of the virus that cause disease in honey bees (5-7). Because iflaviruses spread rapidly in

*Correspondence: Gözde Büşra EROĞLU
Erzurum Technical University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Turkey, 25050
E-mail: gozdebusra.eroglu@erzurum.edu.tr



these insects, they cause deformation, behavioral changes, and death in individuals. These viruses include deformed wing virus (DWV) and sacbrood virus (SBV). So far, there are many studies on DWV and SBV but the knowledge on iflaviruses in general is limited due to the fact that the iflaviruses are found in agricultural and forest pest insects generally asymptomatic (8-12). Infection of agricultural and forest pest insects with the virus is both by horizontal and vertical transmissions (13).

Most of the agricultural and forest pest insects are in the order Lepidoptera (14, 15). Iflaviruses cause infection in these insects alone and or coinfection with other insect viruses (16). Iflaviruses generally reproduce asymptotically in insect larvae of agricultural pests (17). In recent years, with the development of omic technologies, many new infective viruses have been identified from larvae of agricultural and forest pests and genome analysis of many of them has been performed (16,18-24). Genome analysis studies provide valuable information for the evaluation of the relationships between viral isolates in the same family. In this study, the relationships between 14 isolates of iflavirus isolated from agricultural and forest pests, whose whole genome sequences are available on the NCBI database were examined.

Materials and Methods

Kimura-2 Parameter Analysis: To evaluate the relationship between iflaviruses isolated from agricultural and forest pests, those with complete genome sequences available in the NCBI database were used (Table 1). The transition/transversion ratios in the polyproteins of these isolates were determined by the Kimura analysis and the close relationship between them was evaluated. This analysis considered the complete ORF coding for the polyprotein of 14 iflaviruses. Sequences were aligned with the BioEdit program and the distance analysis (Kimura-2 parameter) in the MEGA 11 program was performed.

Phylogeny: The amino acid sequences encoded by the complete polyprotein of the 14 iflavirus isolates were aligned using the program BioEdit (7.1.3.0). For phylogenetic tree analysis, the Jones-Taylor-Thornton (JTT) model with 1000 bootstrap in the Maximum Likelihood method was used to generate a phylogenetic tree using the MEGA 11 program.

Results

Kimura-2 parameter analysis: The transition/transversion distances between the isolates were investigated according to the complete nucleotide sequence of the polyprotein ORF among the isolates that have been detected so far in agricultural and forest pest insects and whose whole genome sequence has been analyzed (Table 2). According to the results obtained, the nucleotide distance between the iflavirus isolates (except *Opsiphones invirae* iflavirus) obtained from insect species that cause damage in the forest area was below 0.5. The distance between *Lymantria dispar* iflavirus Russia1 and Russia2 isolates was 0.002 while the distance between *Lymantria dispar* iflavirus Russia isolates and *Lymantria dispar* iflavirus USA isolate was 0.055. It was found that the nucleotide distance of the iflavirus Turkey isolates of *Thaumetopoea pityocampa*, a very important forest pest, with other forest pests was between 0.347-0.369. While the distance between the iflavirus isolates of *Antheraea mylitta* and *Antheraea pernyi* in the same genus is 0.287, their distance from other forest pests is between 0.349-0.374. However, it is seen that *Helicoverpa armigera* iflavirus isolate which is an important agricultural pest, is closer to the iflavirus isolate obtained from forest pests (0.429-0.466) rather than iflaviruses isolated from other agricultural pests (1.254-1.631). There is an interesting situation among the three viruses isolated from *Spodoptera exigua*. *Spodoptera exigua* iflavirus Spain1 isolate has a value (1.884-1.885) that is quite far from Spain2 and Korea isolates. The nucleotide distance from all isolates

Table 1. Information of the Iflavirus genomes used in the analyzes

Host name	Host family	Pest plant	Origin	Genome size	Accession number	References
<i>Antheraea pernyi</i>	Saturniidae	Forest	China	10176 kb	NC_023483.1	Geng et al., 2014
<i>Antheraea mylitta</i>		Forest	India	9728 kb	MW115117.2	unpublished
<i>Plutella xylostella</i>	Plutellidae	Cabbage	Australia	9623 kb	MN328434.1	unpublished
<i>Plutella xylostella</i>			China	9580 kb	KY435608.1	unpublished
<i>Helicoverpa armigera</i>	Noctuidae	Variety agricultural products	China	10017 kb	NC_033619.1	Yuan et al., 2017
<i>Heliconius erato</i>	Nymphalidae	<i>Passiflora suberosa</i>	Costa Rica	9910 kb	NC_024016.1	Smith et al., 2014
<i>Lymantria dispar</i>	Erebidae	Forest	Russia1	10121 kb	MT753155.1	unpublished
<i>Lymantria dispar</i>			Russia2	9996 kb	MN938851.1	Pavlushin et al., 2021
<i>Lymantria dispar</i>			USA	10044 kb	NC_024497	Carrillo-Tripp et al., 2014
<i>Opsiphones invirae</i>	Nymphalidae	Forest	Brazil	9855 kb	NC_027917.1	Silva et al., 2015
<i>Spodoptera exigua</i>	Noctuidae	Variety agricultural products	Spain1	10347 kb	NC_016405.1	Millán-Leiva et al., 2012
<i>Spodoptera exigua</i>			Spain2	9504 kb	KJ186788.1	Jakubowska et al., 2014
<i>Spodoptera exigua</i>			Korea	9501 kb	NC_023676.1	Choi et al., 2012
<i>Thaumetopoea pityocampa</i>	Thaumetopoeidae	Forest	Turkey	9816 kb	KP217032.1	Jakubowska et al., 2014

except *Opsiphones invirae* iflavirus was also quite far (1.343-1.809). The only virus isolate closely related to *Spodoptera exigua* iflavirus Spain1 was *Opsiphones invirae* iflavirus isolate (0.676) (Table 2).

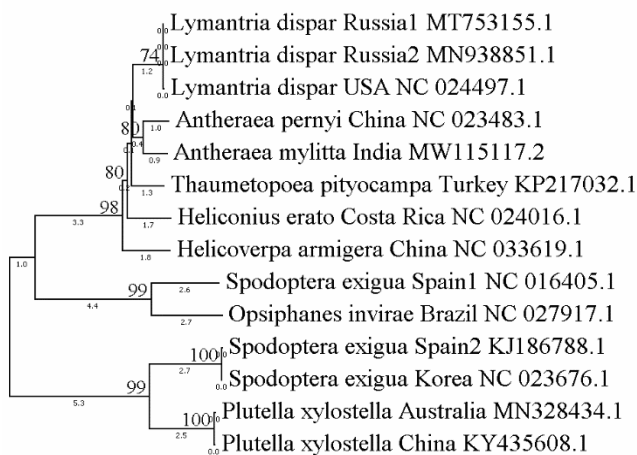


Figure 1. The Immunocytochemical illustration of anti-Beclin-1 and anti-LC-3 staining of HT-29 cells incubated with 0nM, 10nM, 20nM, and 40nM concentration of Bortezomib for 24h.

Phylogeny: As a result of the phylogenetic analysis of the 14 isolates based on their complete polyprotein amino acid sequences, it was seen that with some exceptions, insect viruses of forest pests clustered together while insect viruses of agricultural pests separately clustered together and the results supported the Kimura-2 parameter analysis. Exceptionally, it was observed that the iflavirus isolate from the agricultural pest *Helicoverpa armigera* clustered far away from the iflavirus isolates of other agricultural pests (*Plutella xylostella* and *Spodoptera exigua*), but clustered close to the iflaviruses isolated from forest pests. In addition, it was observed that *Spodoptera exigua* iflavirus Spain1 isolate clustered closely with *Opsiphones invirae* iflavirus isolate instead of other *Spodoptera exigua* iflavirus isolates (Figure 1).

Discussion

The family *Iflaviridae* is newly established and has only one genus (*Iflavirus*) (25). While infective viruses

Table 2. Kimura-2 parameter analysis of iflavirus genomes

Spodoptera exigua Spain1 NC016405.1													
Spodoptera exigua Spain2 KJ186788.1	1,884												
Spodoptera exigua Korea NC 023676.1	1,885	0,015											
Helicoverpa armigera China NC 033619.1	1,349	1,600	1,609										
Lymantria dispar Russia1 MT753155.1	1,350	1,574	1,583	0,433									
Lymantria dispar Russia2 MN938851.1	1,345	1,575	1,581	0,433	0,002								
Lymantria dispar USA NC024497.1	1,353	1,590	1,592	0,436	0,055	0,055							
Thaumetopoea ptyocampa Turkey KP217032.1	1,368	1,592	1,588	0,453	0,348	0,347	0,350						
Heliconius erato Costa Rica NC024016.1	1,392	1,614	1,609	0,466	0,429	0,429	0,436	0,395					
Plutella xylostella Australia MN328434.1	1,809	0,723	0,724	1,615	1,584	1,579	1,578	1,530	1,626				
Plutella xylostella China KY435608.1	1,802	0,730	0,735	1,631	1,582	1,578	1,587	1,531	1,641	0,056			
Antheraea pernyi China NC023483.1	1,360	1,649	1,650	0,453	0,371	0,372	0,374	0,369	0,459	1,615	1,615		
Antheraea mylitta India MW115117.2	1,343	1,599	1,600	0,429	0,349	0,350	0,354	0,366	0,440	1,552	1,549	0,287	
Opsiphanes invirae Brazil NC027917.1	0,676	1,727	1,720	1,254	1,273	1,268	1,262	1,252	1,276	1,758	1,788	1,274	1,255

cause severe infections in beneficial insects, they generally remain asymptomatic in harmful insects (8-12). Therefore, more data are needed on iflaviruses found in agricultural and forest pest insects. In this study, the relationship between iflavirus isolates found in the NCBI database and whose complete genome analysis has been performed so far from pests of the order Lepidoptera was evaluated according to the Kimura-2 parameter and amino acid tree. The Kimura-2 parameter data obtained as a result of the study and the phylogenetic analysis results supported each other. Accordingly, iflaviruses isolated from lepidopteran species, especially forest pests, showed a great clustering with each other. However, some of the iflaviruses isolated from the agricultural pest lepidopteran species were more similar to each other and some of the isolates in the forest group. Although

Helicoverpa armigera and *Spodoptera exigua* are important agricultural pests, are in the same family (Noctuidae), the iflaviruses isolated from them are quite far from each other. *Helicoverpa armigera* iflavirus isolate clustered close to the forest pest *Lymantria dispar*, *Antheraea pernyi*, *Antheraea mylitta*, *Thaumetopoea ptyocampa*, and *Heliconius erato* iflavirus isolates. In addition, *Spodoptera exigua* iflavirus Spain1 isolate is distantly related to other *Spodoptera exigua* iflavirus Spain2 and Korea isolates (16, 18, 19) and showed similarity only to *Opsiphanes invirae* iflavirus isolate. (23). In this study, nucleotide distance analyzes of iflavirus isolates isolated from harmful insects in the order Lepidoptera for the first time were determined using the Kimura-2 parameter. The obtained data supported the addition of a new genus of the *Iflaviridae* family and the inclusion of

Spodoptera exigua iflavirus Spain1 isolate and Opsiphones invirae iflavirus Brazil isolate into a new genus by detecting detailed morphological data.

Declaration of Interest: The author declares that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions: GBE contributed to the study conception, design and laboratory work. Writing the article (GBE). All authors read and approved the final manuscript. GBE; Gözde Büşra Eroğlu

ORCID:

Gözde Büşra Eroğlu  0000-0001-8988-1315

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