6.7 FK506-Binding Protein from *Diaphorina citri* (Hemiptera: Psyllidae)

Hunter W.B. and Shatters, Jr R.

USDA, ARS, U.S. Horticultural Research Lab, 2001 South Rock Rd, Ft. Pierce, FL, USA 34945.

We report the isolation and characterization of the first 11.7-kDa FK506-binding protein from psyllids, *Diaphorina citri*. New strategies in insect and disease management depend on using genes and proteins of an organism against itself, through disruption, down-regulation, or silencing methodologies. To facilitate the rapid develop of these strategies, gene expression libraries have been produced from *D. citri* (Hunter et al., 2005,2006,2008). Mining of these datasets has identified many potential genetic targets for psyllid management. One such protein described a member of the FK506-binding proteins (FKBPs). The FKBPs represent a large gene family in plants and insects that are involved in growth and development [pfam00254]. They are a highly conserved and ubiquitous group of chaperones that bind immunosuppressive proteins. The *D. citri*-FK506BP member had a *calc.* molecular weight of 11.7 kDa, with 109 amino acids in length (Table 1). These proteins are involved in regulation of Calcium ion channels within intracellular membrane systems which is associated with certain pathological states. This means that the movement of salts and water within cell fluids are regulated such that disruption of these channels causes or stimulates cell death. Disruption of genes encoding FKBPs in plants and animals has underlined the importance of this family of proteins in the regulation of cell division and differentiation.

FKBPs, like other immunophilins, are found in all classes of organisms, some of them being highly conserved and others being more specific. These proteins have multiple roles in the cell, the best known as receptors for medically important immunosuppressors. However, the functions of these enzymes are still being elucidated and recent results obtained in plants and animals indicate that these proteins are master regulators in the control of development.

Several FKBPs have been identified in invertebrates, including in the tunicate *Botryllus schlosseri*, the sponge *Suberites domuncula*, in worms *Schistosoma mansoni* and *Caenorhabditis elegans*, as well in several insects, *Drosophila melanogaster*, *Spodoptera frugiperda* (Alnemri et al., 1994), *Manduca sexta*, *Anopheles gambiae* and *Bombyx mori* (Somarelli, et al., 2007). In insects, FKBPs can be separated into several main categories. The first group includes orthologues to human FKBP12 and FKBP13. The second type of insect FKBPs are all similar in sequence and range in size from 38–46 kDa. The third class of insect FKBPs corresponds to potential orthologues of human FKBP52. Much of the empirical work on insect FKBPs has been related to FKBP46, which was first identified in *Spodoptera frugiperda* Sf9 cells (Alnemri et al., 1994). In Lepidoptera, *Manduca sexta*, the FKBP46 is a member of the ecdysone receptor (EcR) complex (Song et al., 1997).

Methods and Materials

cDNA library construction. Adult *D. citri* were collected from citrus seedlings, Evans Properties, for RNA extraction and library construction as in (Hunter et al., 2008). Sequences have been deposited in the public database NCBI (Hunter et al., 2005ab, 2006, Hunter and Hall 2008).

Bioinformatics analyses of Diaphorina citri-FKBP12. The *D. citri*-FKBP12 cDNA [ABG82004.1|110671506] was translated into an amino acid sequence using the ExPASy translate tool. Multiple sequence alignments were generated using the ClustalW alignment tool bundled within the BioEdit program version 7.0.5 (Fig 1)(Hall, 1999). Alignments were performed using the ClustalW default parameters with no gap penalties and using the BLOSUM62 similarity matrix. Identities and similarities, as well as the rate of conservative versus non-conservative amino acid substitutions, between the FKBP12 proteins were calculated using the BLOSUM62 matrix (Henikoff and Henikoff, 1992) (Table 1). Phylogenetics analyses were performed using the Paup package version 4.0. Phylogenetic reconstructions were obtained with both mRNAand protein alignments using maximum parsimony and 1000 bootstrap replicates (Fig 2). (Somarelli and Herrera, 2007). The *D. citri* FKBP506 sequence were determined using cDNA, TBLASTX, and BLASTP, NCBI nr databases. *Diaphorina citri* FKBP12 was most closely related to *Bombyx mori* [114052971], with 84% identities and 91% positive amino acid matches.

Results and Discussion

Here, we report the isolation and characterization of a FK506-binding protein, with a molecular weight of 11.7-kDa (*calc.*) from the Asian citrus psyllid *Diaphorina citri*. The FKBP12, have been shown to have a role as modulators of calcium channel functions (Marks 1996). The *Diaphorina citri* FKBP12 was most closely related to *Bombyx mori* [114052971], with 84% identities and 91% positive amino acid matches. Disruption of this and similar genes may provide unique genetic targets to reduce psyllid survival, thus limiting populations in the field.

Mining of the available genomic information from adult Asian citrus psyllid, *Diaphorina citri*, cDNA libraries (Hunter *et al*) identified a FK-binding protein (FKBP) which functions in many critical pathways needed for psyllid survival. The FKBPs are members of the peptidylprolyl *cis trans* isomerase family of enzymes, PPIases. They are a highly conserved and ubiquitous group of chaperones that bind immunosuppressive macrolides. The best studied proteins from the FKBP's are the 12 kDa FKBPs, which participate in a diversity of cellular functions. In addition members of this family are involved in apoptosis, cell-cycle progression, calcium release, nucleic acid binding and transport of high-molecular-mass receptor complexes. Several proteins that associate with FKBP12 have been identified in insect systems; such as calmodulin and calcineurin homologues in *Bombyx mori* (silkworm), mTOR and ryanodine receptors in *Drosophila melanogaster* and two calmodulin-like proteins in *Manduca sexta* (tobacco hornworm) suggesting that similar FKBP-mediated pathways and interactions exist in insects. Herein we describe the first *D. citri*-FK506BP of a molecular weight of 11.7 kDa, of 109 amino acids. The *D. citri* FK506BP cDNA was also isolated and sequenced using adult whole body tissues. A high degree of similarity among FKBP proteins across many different taxonomic groups has been reported which includes insects, bacteria, fungi, nematodes, tunicates, fish,

plants and mammals and found to be highly conserved. The importance of FK506BP functions may lend it to be a possible genetic target to reduce *D. citri* populations through the use of emerging RNAi technologies in insect management.

References

Alnemri ES., Fernandes-Alnemri T, Pomerenke K, *et al.* 1994. FKBP46, a novel Sf9 insect cell nuclear immunophilin that forms a protein-kinase complex. J. Biol. Chem. 269:30828–834.
Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41:95–98.
Henikoff S, Henikoff JG. 1992. Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA. 89:10915–10919.
Hunter WB, Dowd SE, Katsar CS, Shatters Jr RG, McKenzie CL, Hall DG. 2008. Psyllid biology: expressed genes, *Diaphorina citri*. Insect Science (submitted 07/08/08).
Hunter WB, Dang P, McKenzie CL. 2005 *Diaphorina citri* (Hemiptera) DN465721-DN470410, NCBI.
Hunter WB, McKenzie CL, Shatters RG. Hall DG. 2005. Gene expression in field collected Asian citrus psyllids, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) DN202325-DN201110. NCBI.
Hunter WB, Hunnicutt LE, Hall DG. 2006. Ribosomal proteins of the Asian citrus psyllid, *Diaphorina citri*. 88 Proteins, DQ675535-DQ673441. NCBI.
Hunter WB., Hall DG. 2008. *Diaphorina citri* midgut gene expression, Asian citrus psyllid (Hemiptera: Psyllidae) FK254041- FK260232, NCBI.
Kay JE. 1996. Structure–function relationships in the FK506-binding protein (FKBP) family of peptidylprolyl *cis–trans* isomerases. Biochem. J. 314:361–385.
Marks AR. 1996. Immunophilin modulation of calcium channel gating. Methods 9:177-187.
Somarelli JA, Herrera RJ. 2007. Evolution of the 12 kDa FK506-binding protein gene. Biol. Cell 99:311-321.
Somarelli JA, Coll JL, Velandia A, Marinez L, Herrera RJ. 2007. Characterization of immunophilins in the silkmoth *Bombyx mori*. Archives of Insect Biochemistry and Physiology 65:195-209.
Song Q, Alnemri ES, Litwack G, Gilbert LI. 1997. An immunophilin is a component of the insect ecdysone receptor (EcR) complex. Insect Biochem Mol Biol 27:973–982.

Figure 1. Sequence and structural alignment of FK506 Binding Protein, FKBP, from Asian Citrus Psyllid, *Diaphorina citri*. A multiple sequence alignment among species from insect taxonomic groups shows high similarity among FKBP12 paralogues and orthologues at the amino acid level (**blue**). Critical residues necessary for PPIase activity and FK506 binding, according to Kay (1996), are highlighted in **grey color.**

CLUSTAL W (1.81) multiple sequence alignment

```
[Bombyx_mori]gi|116688016|gb|A      MGVTVETISPGDGSTYPKSGQTVVVHYTGTLTNGKKFDSSRDRGKPFKFRIGKSEVIRGWDEGVAQMSVGERAKL
[Manduca_sexta]gi|6560679|gb|A      MGVTVDTITPGDESTYPKNGQTVVVHYTGTLTSGKKFDSSRDRGKPFKFRIGKGEVIRGWDEGVAKMSVGERAKL
[Diaphorina_citri]gi|110671506      MGVDVETLSPGDGQTYPKPGQVVVVHYTGTLTDGTKFDSSRDRGVPFKFRLGKGDVIKGWDHGIAQLCVGQTAKL
[Bombyx_mori]gi|114052971|ref.      MGVDVETISPGNGSTYPKPGQTVVVHYTGTLQNGKKFDSSRDRGQPFKFTLGKGDVIKGWDQGLAKMSVGERAKL
[Apis_mellifera]gi|66558413|re      MGVDVEVLSPGDGQTYPKTGQTVVVHYTGTLDNGKKFDSSRDRGVPFKFKIGKGEVIKGWDQGVAKMCVGERARL
[Nasonia_vitripennis]gi|156555      MGVNVEVLSPGDGQTYPKTGQTVVVHYTGTLANGKKFDSSRDRGVPFKFKIGKGEVIKGWDQGVAQMCVGERARL
[Tribolium_castaneum]gi|910866      MGVQVDTISPGDGQTFPKTGQTVVVHYTGTLENGTKFDSSRDRGVPFKFRIGKGEVIKGWDEGVAQLSVGQRAKL
[Maconellicoccus_hirsutus]gi|1      MGVQVETISPGDGSTYPKHGQTVVVHYTGTLVDGKKFDSSRDRGTPFKFKLGKGEVIKGWDEGVAQLCVGQRARL
[Drosophila_melanogaster]gi|78      MGVQVVPIAPGDGSTYPKNGQKVTVHYTGTLDDGTKFDSSRDRNKPFKFTIGKGEVIRGWDEGVAQLSVGQSAKL
[Aedes_aegypti]gi|157117168|re      MGVQVVTLAAGDEATYPKAGQVAVVHYTGTLADGKVFDSSRTRGKPFRFTVGRGEVIRGWDEGVAQMSVGQRAKL
[Danio_rerio]gi|41152406|ref|N      MGVEVETITPGDGSTFPKKGQTCVVHYVGSLTDGRKFDSSRDRGKPFKFKIGKQEVIRGWDEGVAQMSVGQRAKL
[Tetraodon_nigroviridis]gi|472      MGVEVETIVPGDGQTFPKKGQRVVVHYVGTLMNGQMFDSSRDRGKPFKFKIGHGEVIRGWEEGVAQMSVGQRAKL
[Xenopus_laevis]gi|27469642|gb      MGVDLETISPGDGRTFPKKGQTCVVHYTGMLQNGKKFDSSRDRNKPFKFKIGRQEVIKGWEEGVAQMSLGQRAKL
[Bos_taurus]gi|118151036|ref|N      MGVQVETISPGDGRTFPKHGQTCVVHYTGTLEDGKKFDSSRDRNKPFKFVLGKKQVIRGWEEGIAQMSIGQRAKL
                                    *** :  : .*:  *:** **  .***.* * .*  ***** *. **:* :*: :**:**:.*:*::.:*: *:*

[Bombyx_mori]gi|116688016|gb|A      TCSPDYAYGGQGHPGVIPPNSTLIFDVELLRLE-
[Manduca_sexta]gi|6560679|gb|A      TCTPDYAYGGQGHPGVIPPNSTLIFDVELLRLE-
[Diaphorina_citri]gi|110671506      TCSPDFAYGSRGHPGIIPPNATLIFDVELLRVEP
[Bombyx_mori]gi|114052971|ref.      TCSPDFAYGSRGHPGVIPPNATLIFDVELLRVE-
[Apis_mellifera]gi|66558413|re      TCSPDFAYGSRGHPGVIPPNAVLIFDVELLKVEP
[Nasonia_vitripennis]gi|156555      TCPPEVAYGPRGHPGVIPPNATLIFDVELLKVE-
[Tribolium_castaneum]gi|910866      TCSPDYAYGSRGHPGIIPPNSTLIFDVELLKVE-
[Maconellicoccus_hirsutus]gi|1      ICSPDYAYGSRGHPGIIPPNSTLIFDVELLKVES
[Drosophila_melanogaster]gi|78      ICSPDYAYGSRGHPGVIPPNSTLTFDVELLKVE-
[Aedes_aegypti]gi|157117168|re      VCSPDYAYGSRGHPGVIPPNATLTFDVELLRVE-
[Danio_rerio]gi|41152406|ref|N      TCTPDFAYGSKGHPGVIPPNATLIFDVELIGLE-
[Tetraodon_nigroviridis]gi|472      ICSPDFAYGSKGHPGIIPPNATLIFDVELLGLE-
[Xenopus_laevis]gi|27469642|gb      TCSPDVAYGATGHPGVIPPNATLIFDVELIRIE-
[Bos_taurus]gi|118151036|ref|N      TVSPDYAYGSRGHPGIIPPNATLIFDVELLKLE-
                                      .*: ***  ****:****:.* *****: :*
```

Table 1. Average size of insect FKBP's (in shading), reduced number of members used, alignments with *D. citri* FK506 Binding Protein, ~11.7 kDa mol. wt. (CLUSTALW (1.81).

Sequence type explicitly set to Protein, Sequence format is Pearson

Sequence 1:	[Diaphorina_citri]gi|110671506	109 aa
Sequence 2:	[Apis_mellifera]gi|66558413|re	109 aa
Sequence 3:	[Tribolium_castaneum]gi|910866	108 aa
Sequence 4:	[Bombyx mori]gi|114052971	108 aa
Sequence 5:	[Maconellicoccus_hirsutus]gi|1	109 aa
Sequence 6:	[Nasonia_vitripennis]gi|156555	108 aa
Sequence 7:	[Bombyx_mori]gi|116688016|gb|A	108 aa
Sequence 8:	[Manduca_sexta]gi|6560679|gb|A	108 aa
Sequence 9:	[Drosophila_melanogaster]gi|78	108 aa
Sequence 10:	[Aedes_aegypti]gi|157117168|re	108 aa
Sequence 11:	[Danio_rerio]gi|41152406|ref|N	108 aa
Sequence 12:	[Xenopus_laevis]gi|27469642|gb	133 aa
Sequence 13:	[Bos_taurus]gi|118151036|ref|N	108 aa
Sequence 14:	[Tetraodon_nigroviridis]gi|472	108 aa

Phylogenetic tree examined with bootstrap with the number of exons in each clade to the right between the brackets. Although the bootstrap support is lower, the phylogenetic cladogram constructed from protein sequences (inset) supported the same general morphology as the one based on mRNA sequences. The overall dendrogram topology based on mRNA sequences supports placement of the *Diaphorina citri* FKBP within the insect clade (after Somarelli and Herrera, (2007).
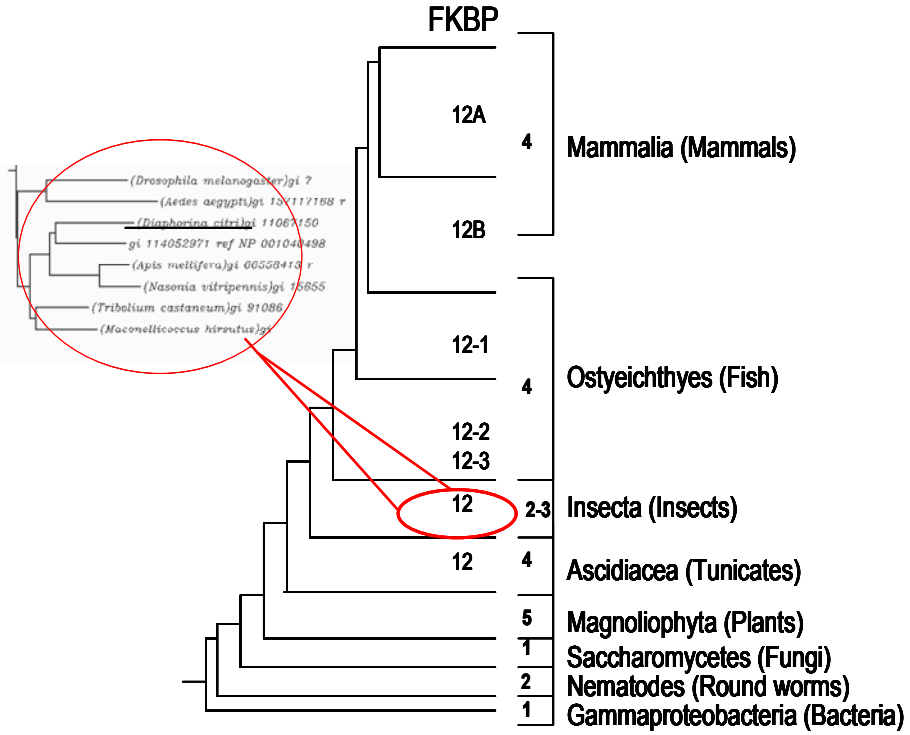
Figure 2. Phylogenetic relationships of FKBP genes using maximum parsimony.