Journal of Invertebrate Pathology 104 (2010) 180–185

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Discovery and effects of Texas *Solenopsis invicta* virus [SINV-1 (TX5)] on red imported fire ant populations

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ARTICLE INFO

Article history:
Received 26 June 2009
Accepted 24 March 2010
Available online 27 March 2010

Keywords:
Colony collapse
Solenopsis invicta
Red imported fire ant
SINV
Picornaviridae
Dicistroviridae
Biological control
Viral isolation
Israeli acute paralysis virus (IAPV)
SINV-1 (TX5) accession No. FJ229495

ABSTRACT

Solenopsis invicta Buren (Hymenoptera: Formicidae), the red imported fire ant is native to South America but has invaded areas of the southeastern US, and parts of Southern California. The *S. invicta* virus-1 (SINV-1) is a positive sense, single-stranded RNA picorna-like virus that only affects *Solenopsis* species. The virus can infect all caste members and developmental stages. Infection of SINV-1 can result in colony collapse in less than 3 months under laboratory conditions. This study screened *S. invicta* colonies from Texas for the presence of SINV through Reverse Transcriptase PCR (RT-PCR). Positive samples were genetically characterized by direct sequencing and compared with known picorna-like viruses. SINV-1 was detected in ant colonies from Smith and Henderson TX counties. Amino acid sequence comparison of SINV-1 (TX5) ORF2 region showed homologies of 96% with SINV-1, 97% with SINV-1A, 17.6% with SINV-2, and 20.7% with SINV-3. In addition, SINV-1 (TX5) was compared to 18 other *Dicistroviridae* viruses. Ant-infecting viruses may provide new approaches to suppressing these important economic pests.

1. Introduction

The "picornavirus-like superfamily" includes *Comoviridae*, *Dicistroviridae*, *Picornaviridae*, *Potyviridae* and *Sequiviridae* which can be distinguished from one another by differences in the positioning of structural proteins and number of genomic segments present (Büchen-Osmond, 2006; Bonning, 2009). Picornaviruses and dicistroviruses are similar because they both possess unenveloped icosahedral virions which are tightly packed into four groups of equilateral triangles that make up the surface of the viral capsid protein (Rueckert, 1991) and differ in their various mechanisms for receptor attachment (Bonning, 2009).

The family *Dicistroviridae* is classified as Group IV insect-infecting viruses which have positive sense, single-stranded RNA genomes of 7.2–9.0 kb long (Mettenleiter and Sobrino, 2008). Currently, there are 12 dicistroviruses (according to the International Committee on Taxonomy of Viruses (ICTV), two additional viruses are awaiting approval (*Homalodisca coagulata* virus-1 (HoCV-1, Hunter et al., 2006) and Israeli acute paralysis virus (IAPV, Maori et al., 2007a); last updated 8/7/08). These viruses infect a variety of insect host orders including Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, and Decapoda (Crustacea) (Hunter et al., 2006; Bonning, 2009). This family consists of a single genus *Cripavirus*, named after the type species Cricket paralysis virus (CrPV) (Büchen-Osmond, 2006), and includes CrPV, Aphid lethal paralysis virus (ALPV), Black queen cell virus (BQCV), *Drosophila* C virus (DCV), Himetobi P virus (HiPV), *Plautia stali* intestine virus (PSIV), *Rhopalosiphum padi* virus (RhPV), Triatoma virus (TrV), and possibly HoCV-1 (Hunnicutt et al., 2006). A proposed new genus *Aparavirus*, named after Acute bee paralysis virus (ABPV), would separate out ABPV, Taura syndrome virus (TSV), Kashmir bee virus (KBV), *Solenopsis invicta* virus-1 (SINV-1), and possibly Israeli acute paralysis virus (IAPV).

Some dicistroviruses were discovered to be major contributors to honey bee mortality. ABPV, KBV, and IAPV are commonly detected in honey bee colonies associated with colony collapse disorder (CCD) (Anderson and Gibbs, 1988; Cox-Foster et al., 2007). Thus, a similar effect may exist in ants, and would be of economic importance if a viral causative agent could be determined for use as a biological control agent. Many dicistroviruses persist in hosts as asymptomatic, unapparent infection until some stressor (e.g. environmental, virus titer, malnutrition, other pathogens or parasites) or external vector causes the virus to transform to a lethal state (Christian and Scotti, 1998; de Miranda et al., 2004; Hashimoto and Valles, 2007; Maori et al., 2007a). In addition, Maori et al. (2007b) found that a small segment of IAPV RNA was able to

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0022-2011/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2010.03.006

integrate into the genome of honey bees through recombination and retrotransposition, which may aid in host viral resistance.

The first dicistrovirus to be isolated from the red imported fire ant, *S. invicta* Buren, has been characterized as the *S. invicta* virus-1 (SINV-1) (Valles et al., 2004). The RNA genome of SINV-1 is 8026 nucleotides long, has a polyadenylated tail, is single stranded, monopartite, and encodes two large non-overlapping open reading frames (ORF) separated by an intergenic region (IGR). The 5′ ORF1 contains genes that code for helicase, cysteine protease, and RNA-dependent RNA polymerase sequences (Valles et al., 2004; 2007), consistent with other dicistroviruses. SINV-1 also has a long untranslated region (UTR) at the 5′ end that is thought to be important for translation, virulence, and encapsidation (Mettenleiter and Sobrino, 2008). The 3′ ORF2 section of SINV-1 contains sequences similar to those of structural proteins, also known as capsid proteins (Valles and Hashimoto, 2008). The unaccompanied genetic material of most positive sense RNA genomes is infectious, becomes more virulent when contained in a viral particle, and subsequently increases in infectivity when transfected into cells (Büchen-Osmond, 2006).

Currently, three *S. invicta* viruses have been described: SINV-1 (Valles et al. 2004), SINV-2 (Hashimoto and Valles 2008), and SINV-3 (Valles and Hashimoto 2009) and are the first on record to infect all caste members and developmental stages of both monogyne and polygyne *S. invicta* colonies (Valles and Strong, 2005). SINV viruses are solely infectious to the *Solenopsis* genus (Valles et al., 2007). SINV-1A has been associated closely with SINV-1 and was determined to be a close genotype of SINV-1 (Valles and Strong, 2005). The SINV-1A partial genome contains 2845 nucleotides and encodes only one large ORF (Valles et al., 2004). SINV-1 and SINV-1A tend to co-infect only 17.5% of *Solenopsis* colonies; however, they are 97% genetically similar to each other (Valles and Strong, 2005). Virulence of these ssRNA viruses in fire ants have proven to be relatively benign or covertly infectious in field studies (Valles et al., 2004), however changes or mutations which alter amino acids could result in increased infectivity, pathogenicity, or transmissibility. Dicistroviruses can be transmitted horizontally (orally) from females to males (Gomirez-Zilber and Thomas-Orillard, 1993), and vertically by transovum (Reinganum et al., 1970) or transovarially (Hatfill et al., 1990). Additionally, virus particles are present in the feces of infected individuals, providing another mechanism of nestmate infect (Bonning, 2009). Hashimoto and Valles (2007) discovered that SINV-1 replicates primarily in midgut tissue and can be transmitted to other colony members by trophallaxis.

Small RNA insect viruses have previously been used to control pest populations, such as CrPV used to control populations of olive fruit fly (Manoussis and Moore, 1987) and *Helicoverpa armigera* stunt virus (HaSV) to control the moth *H. armigera* (Christian et al., 2005). Since *Solenopsis* species are known hosts of SINV (Valles et al., 2007), there is potential for this virus to be used as a biological control agent to suppress *S. invicta* populations. This study identified a variant of SINV-1 in Texas, evaluated the variant's affect on mortality in individual ants, and examined similarities of the variant to SINV-1, SINV-1A, and other dicistroviruses.

2. Methods and materials

2.1. Colony collection

S. invicta colonies were collected in February, March, and April, 2008 from Smith and Henderson counties, Texas. All colonies collected were the polygyne phenotype, with 20–30 queens. Colonies ($n \approx 10{,}000$) were excavated and placed into 18.14 kg collection containers with soil. Brood was collected along with queens. Prior to collection, INSECT-a-SLIP (BioQuip Products, Rancho Dominguez, CA) was administered to the top 7.5 cm of the collection containers to prevent the ants from escaping. Tap water was dripped (~10 ml/min) into the collection containers to separate the ants and their brood from the soil. Rafting ants were removed from the surface of the water and put into clear observation trays (57.5 cm × 41 cm × 14.5 cm) lined with 5 cm of INSECT-a SLIP (BioQuip Products, Rancho Dominguez, CA). Smaller (14 cm × 10 cm × 4 cm) clear boxes with a dried layer of plaster of paris on the bottom (0.64 cm) and a small hole in the lid to allow ants access was used as the ant's brood chamber. All colonies were maintained in the lab (~22.5 °C, 12 h L:D) and received half a Vienna sausage (Libby's, Chicago, IL) once a week with unlimited water.

2.2. RNA extractions and sequencing

RNA was extracted using TRIzol reagent following the manufacture's protocol. Ten individuals were used in each extraction and two extractions were completed for each of the 35 colonies collected. A SuperScript One-Step Reverse Transcriptase PCR (RT-PCR) (Invitrogen, Carlsbad, CA) was performed to produce cDNA. Production of cDNA and RT-PCR was completed using specific primer sets for short segments of SINV-1 (Table 1). The RNA RT-PCR was performed under the following conditions: 45 °C for 50 min, 94 °C for 2 min; denatured 94 °C for 15 s, annealed 56 °C for 15 s, elongated 68 °C for 1:30 min, repeated 35 times; and an additional elongation step of 68 °C for 5 min, and then held at 4 °C (iCycler, BioRad, Hercules, CA).

Samples which resulted in correct band size (~260–650 bp depending on the primer set) were considered virus positive (1% agarose gels). Nucleic acid sequencing used the CEQ™8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA). Samples were not tested for the presence of SINV-2 or SINV-3. A minimum of 10 isolates were sequenced for each primer set and each infected colony was sequenced a minimum of 5 times. Consensus sequences were produced and predicted amino acid, AA, and

Table 1
List of oligonucleotide primers used to sequence SINV-1 and SINV-1 (TX5). Primers with (*) were designed in the Bextine Lab, others were acquired from Valles and Strong (2005).

Designation	Oligonucleotide (5′ → 3′)
p58	GCGATAGGTTAGCTTTAAGTACAATTGGTG
p59	TCCCAATGTGCAATAAACACCTTCA
p62	GGAAGTCATTACGTGGTCGAAAACG
p63	CGTCCTGTATGAAAACCGGTCTTTACCACAGAAATCTTA
p102	CGCCTTAGGATTCGTTAGATACTCACCCG
p114	CTTGATCGGGCAGGACAAATTC
p116	GAACGCTGATAACCAATGAGCC
p117	CACTCCATACAACATTTGTAATAAAGATTTAATT
p118	CCAATACTGAAACAACTGAGACACG
p137	GTCACATCACGTCGGTGTCGT
p188	CTTAATTGTAATTTACTTGAATATGCGTTTGC
p189	GTATCTAACGAATCCTAAGGCGGATTG
p190	CAATCCGCCTTAGGATTCGTTAGATAC
p191	CGGATCTTATGAGTGAAGACACACCAG
p193	CAACCTCTGCTTCCCACGCAC
p221	GATGGTCTCGACCAAATGATATGGAG
p222	ATGAAGATATGAAGGTGTTTATTGCACATTG
p341	CACATAAGGGATATTGTCCCCATG
p343	TGGACGAGACGGATCTTATGAGTG
SINV-1 F1*	CAACCTCTGCTTCCCACGCAC
SINV-1 R1*	AAGCTGGGTGGCAAGTTAGA
SINV-1 F2*	TGTTGTCCTCGATCAATTCG
SINV-1 R2*	CCATACAGGCAATTACCCCA
SINV-1 F3*	ACTCCCACCCCTGAAGATTT
SINV-1 R3*	CTGGACGAGACGGATCTTATG
SINV-1 F4*	CCTGGACGAGACGGATCTTA
SINV-1 R4*	GGTGTCAATGATGCATCTGGG