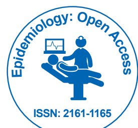

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Short Review on Epidemiology and characterization of Taxon Negevirus

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Abstract

The most recent taxon described Negevirus is made up of a broad collection of viruses that are only found in insects, including mosquitoes and phlebotomine sandflies. In this study, nearly full-length sequences of 91 novel negevirus isolates collected in Brazil, Colombia, Peru, Panama, the United States, and Nepal were subjected to extensive genetic characterization, molecular, epidemiological, and evolutionary analysis. With roots connected to three plant virus genera, we showed that these arthropod-restricted viruses are clustered in two major evolutionary groups (Cilevirus, Higrevirus and Blunevirus). The majority of negeviruses lack distinct host associations, according to molecular investigations; instead, they exhibit considerable genetic variability, a broad host range, and cross-species transmission. The information reported here also identified two arthropod-restrictive viral species and five unique insect-specific viruses, previously proposed as Nelorpivirus and Sandewavirus are separate genera. Our findings contribute to a better understanding of this group of insect-specific viruses' molecular epidemiology, evolution, taxonomy, and stability.

Keywords: Negevirus; phlebotomine; pseudococcid; Cilevirus

Introduction

The species Negevirus has been isolated from mosquitoes and phlebotomine sand flies across the Americas, Europe, Africa, and Asia. It is a monophyletic taxon of non-segmented, positive-sense ssRNA viruses. Egevirus particles are spherical in shape and range in size from 45 to 55 nm. The widely distributed geographically, the wide host range among biting Diptera, and the relatively high natural infection rates in some mosquito species are three intriguing biological traits of the known nege viruses. It has been suggested that the tax on Negevirus can be divided into the Nelorpivirus and Sandewavirus genera based on their evolutionary relationships [1]. New negevirus isolates from mosquitoes and phlebotomine sand flies collected in Brazil, Colombia, Nepal, Panama, Peru, and the United States were examined in the current study. Four novel negevirus species were discovered, and they clump together into two main phylogenetic groupings that correspond to the two above-mentioned proposed genera. A pool of 12 mealy bugs (Paracoccus marginatus), a helipterum pseudococcid that feeds on hibiscus bushes, was also home to a fifth unique negevirus.

Mini Review

This virus is phylogenetically more closely related to plant viruses in the family's Higrevirus, Blunevirus, and Cilevirus and is genetically distinct from nelorpiviruses and sandewaviruses. These results support earlier discoveries that the negeviruses are connected to various species of plant viruses both genetically and evolutionarily [2].

Method

In this investigation, 149 viruses or viral sequences were employed. Their host source, host names, strain names, GenBank accession numbers, taxonomic classifications, isolation year, and geographic locality are all listed. Of the viruses, 58 had already been characterised; details on them were gleaned from GenBank and earlier publications. [3] The remaining 91 viruses were received from virus repositories and sequenced at the Instituto Evandro Chagas (IEC), Ministry of Health, Ananindeua, Para, Brazil, or at the University of Texas Medical Branch (UTMB). In that lab, IEC viruses were discovered in insects gathered during fieldwork in northern Brazil (Amazon Basin). The virus from UTMB was acquired from samples taken during field research in the United States or from material. The staff of the World Reference Center for Emerging Viruses and Arboviruses receives samples provided from other laboratories for diagnostic research. Previous papers described the techniques used for the latter 91 viruses' isolation and preliminary identification [4].

Extraction of RNA

The 91 viruses that were sequenced in our investigation were produced as virus stocks in cultures of C6/36 cells, and the RNA was then extracted as described below. RNA samples were created using the Qiamp RNA micro kit for viruses that were sequenced at UTMB (Qiagen). The Trizol reagent RNA purification method was employed to prepare the Brazilian viruses for RNA extraction.

Negevirus Stability

A high-tittered stock of each virus was introduced to 9.0 ml of a 10 percent sucrose solution together with 1.0 ml of a representative negevirus (Piura, strain EVG 7-47), an alphavirus (Sindbis strain Eg 339), and a flavivirus (West Nile strain NY305-99) to assess their stability. The virus-sucrose solutions were maintained at 28 °C in an incubator, and their infectiousness was frequently tested. For the Sindbis and West Nile virus solutions, samples (0.5 ml) of the virus sucrose solutions were taken initially (day 0), and then every day for 7 straight days. For seven days straight, aliquots of the Piura sample were taken, and subsequently they were taken sporadically until day 76. In 24-well microplate cultures of Vero, the viability (infectivity) of each virus was assessed by titration in duplicate four times [5]. Each daily sample was serially diluted ten times in phosphate-buffered (pH 7.4) saline solution that contains 10% foetal bovine serum. Four wells received 100 uL of each dilution after being mixed. After two hours

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of incubation, maintenance media was added to the cultures. For the following seven days, each plate was checked daily under an inverted tissue culture microscope for signs of viral cytopathic effect (CPE). Using the Reed and Muench formula as per Hsiun's description, the tissue culture infectious dose50 endpoint (TCID50) for each sample was computed.

Genome Sequencing

The Illumina HiSeq, MiSeq, and Ion Torrent equipment' parallel sequencing and Ion Torrent semi-conduction procedures, respectively, were used to extract the virus isolates' genome sequences. In a nutshell, for the Ion Torrent, library preparation, emulsion PCR, and sequencing were carried out as previously described after a complementary DNA was produced from the viral RNA using random hexamer primers. Viral RNA (0.9 g) was fragmented for the HiSeq Illumina technique by incubation at 94 °C for eight minutes in 19.5 l of fragmentation buffer (Illumina 15016648). Using an Illumina TruSeq RNA v2 kit and adhering to the manufacturer's instructions using the 250 paired-end technique, a sequencing library was created from the sample RNA. Regarding the MiSeq illumination technique. A highly effective technique was developed that included rRNA removal after which, using the previously outlined quick, ligation-free cDNA synthesis method, directed RNA-seq libraries are created (Pease and Sooknanan, 2012) [7]. A denovo method implemented in the Mira Software was utilised to combine the data produced by MiSeq and Ion Torrent in order to find potential reference genomes. Following that, the raw data (reads) were mapped using the Newbler algorithm against the selected sequence.

Genome Characterization

Based on genetic characteristics such genome size, genome organisation, and encoded proteins, virus genomes have been classified. Using Geneious version 9, all genomes were quickly examined for the presence of ORFs and annotated. The InterProScan was used to examine protein domains, and the Geneious work package was deployed. Using Geneious and the NetNGly, conserved motifs, cysteine residues, and N possible glycosylation sites were also evaluated.

Genetic Variability

Nucleotide and amino acid sequence alignments were used to assess intra- and intergroup genetic differences, which showed the existence of at least two major groups. Group I included, whereas group II was represented by WALV, GANV, DEZV, SANV, BIRV, and TANV. Nucleotidic and amino acid sequence similarities among these negeviruses varied from 14.8% to 78.6% and 47.8% to 70.8%, respectively, in terms of genetic similarity. Additional analyses using the box plot method showed that each viral group (here presumed to represent virus species) had discrete graphical dispositions and that there were no overlapping groups between the inter- and intra-group areas. The two main groups' box plots and each virus species' separate box plots Three proteins that were encoded by the viral mRNA in various ORFs could be found in the majority of viruses. The viral polymerase protein is encoded by the largest ORF (ORF1), while membrane proteins and glycoproteins are produced by the middle and small ORFs, respectively [8]. The high conserved domains of VMeTrfase, Ribosomal RNA methyltransferase FtsJ domain, and RdRp were linked to conserved areas found in ORF 1.

Discussion

The genomic organisation of the examined viruses is depicted graphically based on the number of ORFs and anticipated protein domains in ORF2, the domain DiSB, a putative virion glycoprotein, and in ORF3, the SP24 domain, characterised and identified as a functional membrane protein domain. An experiment was conducted to compare the stability (infectivity) of a representative negevirus (Piura) held at 28 °C in a 10 percent sucrose solution with the stability of a representative alphavirus (Sindbis) and flavivirus (West Nile) held under the same conditions. This comparison was made because negeviruses share some genetic similarities with some plant viruses. The WNV- sucrose solution had an initial infectivity titer of 109.0 TCID50/ml, but it quickly decreased and by day 4 there was no virus present. The initial virus titer in the Sindbis-sucrose solution was 107.8 TCID50/ml, but no infectious virus was found after day 6.

Conclusion

The Sindbis-sucrose solution displayed a similar but marginally longer survival pattern. In comparison, the Piura-sucrose solution's starting titer was 1010.0 TCID50/ ml and it decreased over the course of the following 76 days relatively gradually. Although we still know very little about the ecology of viruses in the negevirus taxon, there are a few things that can be noted. First off, negeviruses are geographically quite widespread and can be found in both tropical and arctic climates. Field-collected mosquitoes (Culicidae) and phlebotomine sandflies (Phlebotominae) with origins in North and South America, Europe, the Middle East, Asia, and Pacific islands have been used to cultivate isolates of these viruses. Numerous negevirus varieties are anticipated to be discovered given their diversity and vast geographic dispersion. Second, among Diptera, negeviruses have a diverse spectrum of hosts. Although only sandflies and mosquitoes have been known to carry the aforementioned negeviruses, other types of insects may also naturally harbour these viruses.

Acknowledgement

None

Conflict of Interest

None

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