Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **43** Issue-**1 Year 2022** Page **127:135***

Clinical isolates of Anantapuramu for the protein E isolation of the dengue virus

C M Anuradha[1,2]*, K. Chandravathi[6], Gangadharam Poli[1], Suresh Kumar Chitta[1], Madhusudana Pulaganti[3], Prasad Reddy S Hari[4] Chandraprakash Chinta[5]

[1]Department of Bio-Chemistry, Sri Krishnadevaraya University, Anantapuramu, Andhrapradesh, India-515003.

[2] Dept of Biotechnology, Sri Krishnadevaraya University, Anantapuramu, Andhrapradesh, India- 515003.

[3]Multidisciplinary Research Unit, SV Medical College, Tirupati, Andhrapradesh, India-517507

[4]Dept of Biotechnology,,Koneru Lakshmaiah, Education Foundation,Guntur,Andhrapradfesh,India-522502

[5]Department of Zoology, Sri Venkateswara University, Tirupati, Andhrapradesh, India- 517503.

[6]Asst. Professor, Dept. of Forensic Medicine, Sri Venkateswara Medical College, Tirupati.

***Corresponding author**

Dr.C.M.Anuradha

Assistant Professor Dept of Biotechnology Sri Krishnadevaraya University

Anantapur Andhrapradesh

Email: anuradha.priyanka@gmail.com

Article History

Received: 11 Sep 2022

Revised: 26 Oct 2022

Accepted: 10 Nov 2022

ABSTRACT

In theclinical samples from the state of Anantapuramu, the dengue virus serotype 2 protein E gene was found. It was established that the protein E gene was present in the models by using RT-PCR and cellular isolates. There has been one new sub strain discovered that are akin to the hermits described in previous investigations. An investigation of the epidemiology of the isolated strain was conducted using a phylogenetic analysis of the strain.

Keywords: Dengue Virus, Endemic, PCR, Viral Protein E, Viral serotyping

INTRODUCTION

A fast epidemiological development over the preceding two decades has resulted in dengue fever emerging as one of the most remarkable arboviral diseases in recent history(Wu et al. 2021). It is a serious public health hazard around the globe, with tropical and subtropical areas being especially sensitive to its effects(Leitmeyer et al. 1999; Campione-Piccardo et al. 2003). This illness is frequent among tropical nationalities, with severe signs occurring in people of all ages and races(Holmes and Twiddy 2003). When it comes to dengue fever (DF), there is a wide spectrum of illness intensities that vary from moderate to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)(Guha-Sapir and Schimmer 2005; Nogueira et al. 2005). Data gathered from previous

epidemiological studies indicates the seroprevalence of dengue virus serotypes, which include those of the dengue virus serotypes DENV 1-5, in which there are more distinct evolutionary forms depending on geographic structures(Nishiura and Halstead 2007).

In a number of different methodological techniques, the mutation rates of the dengue virus have been determined, and they have been shown to be similar to the evolutionary rates of other RNA viruses via the usage of the envelope protein E(Zheng et al. 2010). As a result, we believe that dengue serotypes should always be researched in a quantifiable way because there is increasing reports that their epidemic potential outcomes differ and that occurrences of secondary infection with different strains oftenly result in higher morbidity and mortality rates due to more severe forms of the infection(dos Santos Soares et al. 2017). Moreover, the causes for the diversity of dengue serotypes are still a riddle. Some researchers believe that the five serotypes developed as a result of different zoonotic transmissions from animals to humans(Clements et al. 2010).

It is possible that DENV progenitors have acclimated to the new host and that positive selection signals have appeared across the DENV genome if this is the scenario(Fried et al. 2010). A large amount of genetic variability exists in RNA viruses as a result of the inherently high mutation rate linked with RNA-dependent RNA polymerase, their rapid replication rates, and their massive population sizes. The existence of five antigenically serotypes of the dengue virus is the most visible manifestation of this genetic heterogeneity in dengue virus(Pawitan 2011). Earlier to the advent of gene sequence information, it was acknowledged that hereditary variety existed within each serotype. However, the discovery of gene sequence information changed that perception(Dash et al. 2011). The development of comparative gene sequence analysis, on the other hand, has permitted the deconstruction of the genetic composition of dengue virus communities as well as the clarification of the mechanisms governing virus transmission(Murray et al. 2013).

It was one of the research goals to conduct a comprehensive study between the development of a dengue virus subtype isolate's E genetic sequence and the emergence of the protein E genetic sequence of dengue virus serotype 2 in this study. In this study, we investigated the segment of the population complexities of outbreak genetic sequences gathered over the previous few decades, using a huge proportion of available to the public sequence data accumulated over the previous few decades. We also calculated evolutionary variables that are essential to understanding the advancement of the DENV 2 serotype. On the other hand, we investigated the role of adaptation evolution in the formation of DENV serotypes and the variety of selecting factors throughout the envelope gene's span.

EXPERIMENTAL METHODOLOGY

Sample Collection and viral characterizations

Clinicians at the Government Hospital in Anantapuramu, India, collected clinical samples from patients who had clinically confirmed dengue fever and brought them to the hospital for testing and analysis. The clinical samples contained acute-phase dengue infections. All samples and collections are sought after in line with the regulations of the institution and the university (Sri Venkateshwara University and Sri Krishnadevaraya University), and they are all obtained in compliance with appropriate ethical standards. It was necessary to get the samples by venipuncture, which were subsequently transported in clinical standards to the Department of Virology laboratory at Sri Venkateshwara University in Tirupati, India, where they were examined. The virus particles are separated from the collected induvial blood by centrifuging it for 10 minutes at 2000 rpm at 4^{o}C for 10 minutes. The virus particles are then removed from the collected induvial blood. In order to conduct additional testing, plasma samples were kept at –80°C until they were needed. The clinical criteria for diagnosing dengue fever were created in line with

the case definitions published by the World Health Organization (WHO), Geneva, Switzerland(Nogueira et al. 2005; Gupta et al. 2012).

Cell culture for Virus cultivation and analysis

Host cells such as Vero cell lines (African green monkey kidney cells) obtained from the American type culture collection (ATCC CCL-81) were used to cultivate the viral isolates. Eagle's minimum essential medium (MEM) supplemented with 5% fetal bovine serum is used in this experiment to develop the cells, which are then harvested. As part of the preparations for long-term usage, the serum concentration in maintenance medium (MM) was decreased to 1.5 %. These cells were grown for a total of 24 hours at 37^{o}Cin a humidified incubator with a 5 % CO_2 flow in a humidified incubator. A 1:10 dilution of viral stock Vero cells ($5x10^4$ ml^{-1}) was introduced into the cells, which were then allowed to proliferate for seven days after the viral particles were withdrawn. The cells were then transfected with the viral particles after they achieved maximum confluence. After scraping the virus particles from the cell lines, they were maintained frozen at -80^{o}C until they were required for further analysis. Three times under each circumstance, the virus was passaged three times, with each passed utilizing a 1:10 dilution of the viral stock from the previous transit, in order to guarantee that the infection remained constant. Viral RNA was extracted from cell debris samples using the QIAamp Viral RNA minikit (Qiagen, Germany), which was performed in accordance with the manufacturer's instructions. The RNA samples were either stored at -70°C or used for RT-PCR as soon as they were extracted, depending on their quality.

Gene extraction from virus and DNA preparation for complementary sequencing

After the RNA has been extracted from the virus particles, the samples are subjected to an expression-based analysis using reverse transcriptase polymerase chain reaction (RT-PCR). The complementary DNA (cDNA) was synthesized by the reverse transcription-polymerase chain reaction (RT-PCR) approach, which used randomized primer sets under optimal PCR conditions. Once the cDNA was obtained, it was kept at -20^{o}C until the Polymerase chain reaction could be performed (PCR)

Polymerase Chain Reaction for gene amplifications and E Protein genes sequencing

Primers were generated and received from Sandor Life Sciences Resources in Hyderabad, India. With the use of these gene specific primers, it is possible to amplify the protein E genes from the viral cDNA and its nearby region using PCR. The PCR reaction is carried out under optimal circumstances with both forward and reverse primers being employed. Amplification of sequences is followed by DNA electrophoresis to determine if the PCR product is in the desired conformation (or not). They are then treated to Exo SAP purification once they have been acquired from the amplification product. PCR products are authorized to undergo sequencing using the ABI Big Dye Terminator V 3.0 Kit, which is performed in accordance with the manufacturer's recommendations. In order to resolve all of these products, DNA electrophoresis was performed using an ABI 3730xl capillary sequencer.

Phylogenetic based epidemiological analysis

The sequences that were collected were then examined in order to evaluate the epidemiological research. The transcripts are aligned for the conservative and variable nucleotides using the NCBI nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with the prior sequences listed in the ncbi database (https://www.ncbi.nlm.nih.gov/) for extremely comparable sequences. It was decided to use the alignments that had been acquired for the building of the phylogenetic tree that would be based on the rapid minimum evolutions. All of the sequences are aligned further utilizing the MEGA software package in conjunction with the crustal W programme in order to achieve the least amount of variance and the

most conservative sequence evaluation and representations possible. With the help of the network format file generated from the NCBI blast data, the resultant tree was further evaluated for genetic distances and representation before being included in ITOL (https://itol.embl.de/login cgi).

RESULTS AND DISCUSSIONS

In this investigation, we obtained blood samples from 200 patients ranging in age from 12 to 18 years, all of whom were displaying symptoms of dengue fever at the time of sample collection, which include skin rashes, aches followed by nausea. When we did the diagnosis on these patients, we observed that 33 of individuals tested positive for the DENV-2 virus. After careful consideration of the seriousness of the patients, the most severe strain of DENV-2 was chosen for this study. The characterization of envelop protein E was then carried out. Following a series of purification procedures, the collected samples are injected with viral particles and incubated in the Vero cell lines for a total of seven days for the multiple cultures. We were able to consistently infected the cell lines, and we were able to extract viral RNA from the develop important samples for further cell line growth. The quantities of the recovered and processed RNA were measured using the nanodrop technique, since the viral load was anticipated to be extracted in lower yield (about 10 pM) and therefore recovered in lower yield. We attempted to replicate the gene using RT-PCR, which was predicated on the customized forward and reverse primers listed in Table-1, and we were successful in obtaining two products, one for each of the primers employed. According to the DNA gel electrophoresis findings for the complementary DNA (cDNA) produced from PCR shown in figure -1, the band at 248bps indicates the replicated cDNA of the DENV-2 virus. We next used sanger-based sequencing to examine the features of the obtained cDNA, and NCBI data set-based sequence alignments confirmed that the transcribed cDNA corresponded to the DENV-2 (dengue virus serotype-2) variety, as shown in the sequences in figure-2. These segments, which were deduced from the reverse and forward primers and assigned DF-2 (Forward) and DR-2 (Reverse), were then decided to submit to the National Center for Biotechnology Information (NCBI), where the accession numbers for DF-2 were OL441604 and DR-2 were OL444951, respectively, and were fruitfully published. Figures 3(a) and 3(b) show the top five sequences for conservation and variable sequences, accordingly. Figures 3(a) and 3(b) depict sequence characteristics for conservation and varied sequences. Figure 3(c) depicts sequence features for the conserved and variable sequences in the top 5 DENV viral protein E sequences (b).

Subsequently, we ran epidemiological analyses for the corresponding sequences, which included DF-2 and DR-2, and received positive findings, with DF-1 showing a clade that was identical to 99.8 percent of the clade sequences for the protein E of the DENV serotype 2 viral features. While the DR-2 revealed a novel variation react by generating a new clade that was 99 % compatible with sequencing data with the DENV-2.

Dengue fever is predicted to affect 2.5 billion people worldwide, putting them at risk of contracting the disease(Roy and Bhattacharjee 2021). A wide range of well-described clinical diseases caused by dengue viruses, such as DF, which is a clinical illness that is often characterized by increased vascular permeability and can advancement to DSS and DHF if the capillaries are becoming engorged. Dengue viruses are also responsible for transmission of dengue fever(Rasheed et al. 2013). Dengue fever outbreaks may have a substantial negative effect on the economy and health of any nation. Viruses of the dengue virus serogroups 1–5 were found to be spreading across Southeast Asia, Africa, and the Americas, marking a significant shift from the scenario 20 or 30 years earlier(Kumar et al. 2010). Taking into consideration epidemiological variance is critical when developing an effective monitoring and preventive programme for any illness(Guzman et al. 2012).

It was observed that all of the viruses studied belonged to the Indian subcontinent and other tropical After performing a phylogenetic analysis of 27 DENV-2 E gene sequences gathered between 2007 and 2017, it was shown that all of the viruses examined belonged to the lineage of the cosmopolitan genotype that originated in the Indian subcontinent and other tropical nations(Murhekar et al. 2019). Even though there were considerably more DF samples than DSS samples among the samples obtained, no differentiating feature in the E gene sequences could be found to differentiate between DF and DSS samples(Dhal et al. 2020). Following the removal of 20 isolates that were closely associated to a prior Asian strain and included isolates from southern India, the remaining 2 isolates formed a separate monophyletic clade, as represented in figure-4 (a), that was different from the previously mentioned Asian strain. In the DENV-2 whole genome analysis, the same clade got 100 percent bootstrap support, further boosting the credibility of the clade for the other sequence, as demonstrated in Figure 4 (b). There were 27 DENV-2 isolates in this monophyletic clade, all of which were recovered from sera collected in the southern portion of India (Anantapuramu, Andhra Pradesh State) in 2017 and 2018. All of the isolates were recovered from sera collected in Anantapuramu, Andhra Pradesh State, in 2017 and 2018. These two viruses exhibited the greatest nucleotide similarity (98.8-99.5 percent) to viruses that had been reported in India and other areas of the globe between 2007 and 2017, as well as to viruses that had been reported in other parts of the world during the same time.

CONCLUSIONS

A significant public health issue in our nation is dengue fever, and effective control measures must be put in place as soon as possible to avoid a new pandemic of epidemic proportions. We have all built a level of tolerance to DENV-1 to 5 in the population, and it has the possibility to become an outbreak strain in the future. Preventative actions must be implemented in a timely and appropriate manner in order to prevent a future pandemic from occurring. In order to plan successful management of the virus or the generation of new vaccines, epidemiologists and virologists may find it useful to investigate these types of issues. It is recommended that future efforts be focused on the development of improved laboratory-based surveillance technology that can identify the beginning of impending dengue epidemics. Thus, the research underlines the role played by viral mutation in the dissemination of dengue to areas where the illness has not previously been a significant public health burden, as well as the generation of new viral strains that may be linked with severe outbreaks.

Acknowledgements

Authors are thankful to authorities of Sri Krishnadevaraya University and Government Medical College Anantapur, Andhrapradesh, India to complete this work.

References

1. Campione-Piccardo J, Ruben M, Vaughan H, Morris-Glasgow V (2003) Dengue viruses in the Caribbean. Twenty years of dengue virus isolates from the Caribbean Epidemiology Centre. The West Indian medical journal 52 (3):191-198
2. Clements DE, Coller B-AG, Lieberman MM, Ogata S, Wang G, Harada KE, Putnak JR, Ivy JM, McDonell M, Bignami GS (2010) Development of a recombinant tetravalent dengue virus vaccine: immunogenicity and efficacy studies in mice and monkeys. Vaccine 28 (15):2705-2715
3. Dash P, Sharma S, Srivastava A, Santhosh S, Parida M, Neeraja M, Subbalaxmi M, Lakshmi V, Rao P (2011) Emergence of dengue virus type 4 (genotype I) in India. Epidemiology & Infection 139 (6):857-861

4. Dhal A, Kalyani T, Ghorai S, Sahu NK, Jana SK (2020) Recent development of electrochemical immunosensor for the diagnosis of dengue virus NSI protein: A review. Sensors International:100030
5. dos Santos Soares RdO, Bortot LO, van Der Spoel D, Caliri A (2017) Membrane vesiculation induced by proteins of the dengue virus envelope studied by molecular dynamics simulations. Journal of Physics: Condensed Matter 29 (50):504002
6. Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Yoon I-K, Jarman RG, Green S, Rothman AL, Cummings DA (2010) Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. PLoS neglected tropical diseases 4 (3):e617
7. Guha-Sapir D, Schimmer B (2005) Dengue fever: new paradigms for a changing epidemiology. Emerging themes in epidemiology 2 (1):1-10
8. Gupta E, Mohan S, Bajpai M, Choudhary A, Singh G (2012) Circulation of Dengue virus-1 (DENV-1) serotype in Delhi, during 2010-11 after Dengue virus-3 (DENV-3) predominance: a single centre hospital-based study. Journal of vector borne diseases 49 (2):82
9. Guzman MG, Alvarez A, Vazquez S, Alvarez M, Rosario D, Pelaez O, Cruz G, Rodriguez R, Pavon A, Gonzalez A (2012) Epidemiological studies on dengue virus type 3 in Playa municipality, Havana, Cuba, 2001–2002. International Journal of Infectious Diseases 16 (3):e198-e203
10. Holmes EC, Twiddy SS (2003) The origin, emergence and evolutionary genetics of dengue virus. Infection, genetics and evolution 3 (1):19-28
11. Kumar K, Singh PK, Tomar J, Baijal S (2010) Dengue: epidemiology, prevention and pressing need for vaccine development. Asian Pacific Journal of Tropical Medicine 3 (12):997-1000
12. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, Chacon d, Ramos C, Rico-Hesse R (1999) Dengue virus structural differences that correlate with pathogenesis. Journal of virology 73 (6):4738-4747
13. Murhekar M, Joshua V, Kanagasabai K, Shete V, Ravi M, Ramachandran R, Sabarinathan R, Kirubakaran B, Gupta N, Mehendale S (2019) Epidemiology of dengue fever in India, based on laboratory surveillance data, 2014–2017. International Journal of Infectious Diseases 84:S10-S14
14. Murray NEA, Quam MB, Wilder-Smith A (2013) Epidemiology of dengue: past, present and future prospects. Clinical epidemiology 5:299
15. Nishiura H, Halstead SB (2007) Natural history of dengue virus (denv)—1 and denv—4 infections: reanalysis of classic studies. The Journal of infectious diseases 195 (7):1007-1013
16. Nogueira RMR, Schatzmayr HG, De Filippis AMB, Dos Santos FB, Da Cunha RV, Coelho JO, De Souza LJ, Guimarães FR, De Araújo ESM, De Simone TS (2005) Dengue virus type 3, Brazil, 2002. Emerging infectious diseases 11 (9):1376
17. Pawitan JA (2011) Dengue virus infection: predictors for severe dengue. Acta Med Indones 43 (2):129-135
18. Rasheed S, Butlin R, Boots M (2013) A review of dengue as an emerging disease in Pakistan. Public health 127 (1):11-17
19. Roy SK, Bhattacharjee S (2021) Dengue virus: Epidemiology, biology, and disease aetiology. Canadian Journal of Microbiology 67 (10):687-702

20. Wu T, Wu Z, Li YP (2021) Dengue fever and dengue virus in People's Republic of China. Reviews in Medical Virology:e2245
21. Zheng A, Umashankar M, Kielian M (2010) In vitro and in vivo studies identify important features of dengue virus pr-E protein interactions. PLoS pathogens 6 (10):e1001157

Table 1: Primer details for Polymerase Chain Reaction

Primer Code	Orientation	5' to 3' direction	Location of genome
D2ES2F	Forward	GGATTATTTGGAAAGGGAGG	1229-1248
D2ES2R	Reverse	GTCCCCTTCATATTGTAC	1906-1925

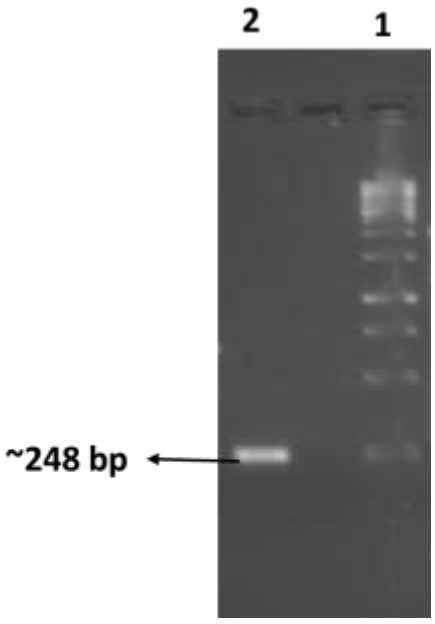

Figure 1: DNA electrophoretic gel images showing (1) The 1kbp DNA ladder and (2) Query sample sequences showing ~248bp

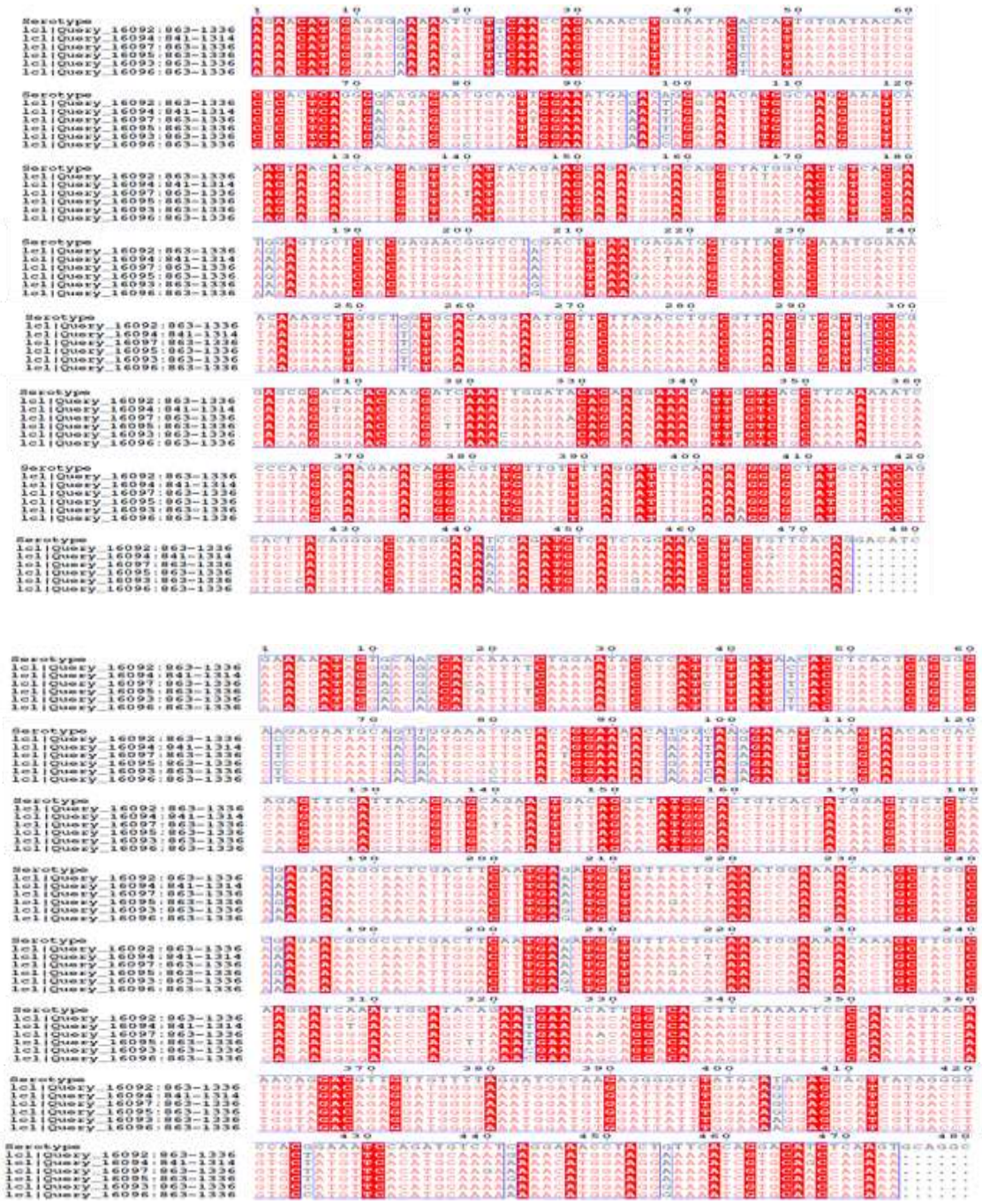

Figure 2: Blast data for the sequences (a) DF-1 and (b) DR-1 with conservative and variant sequences in the query genes.

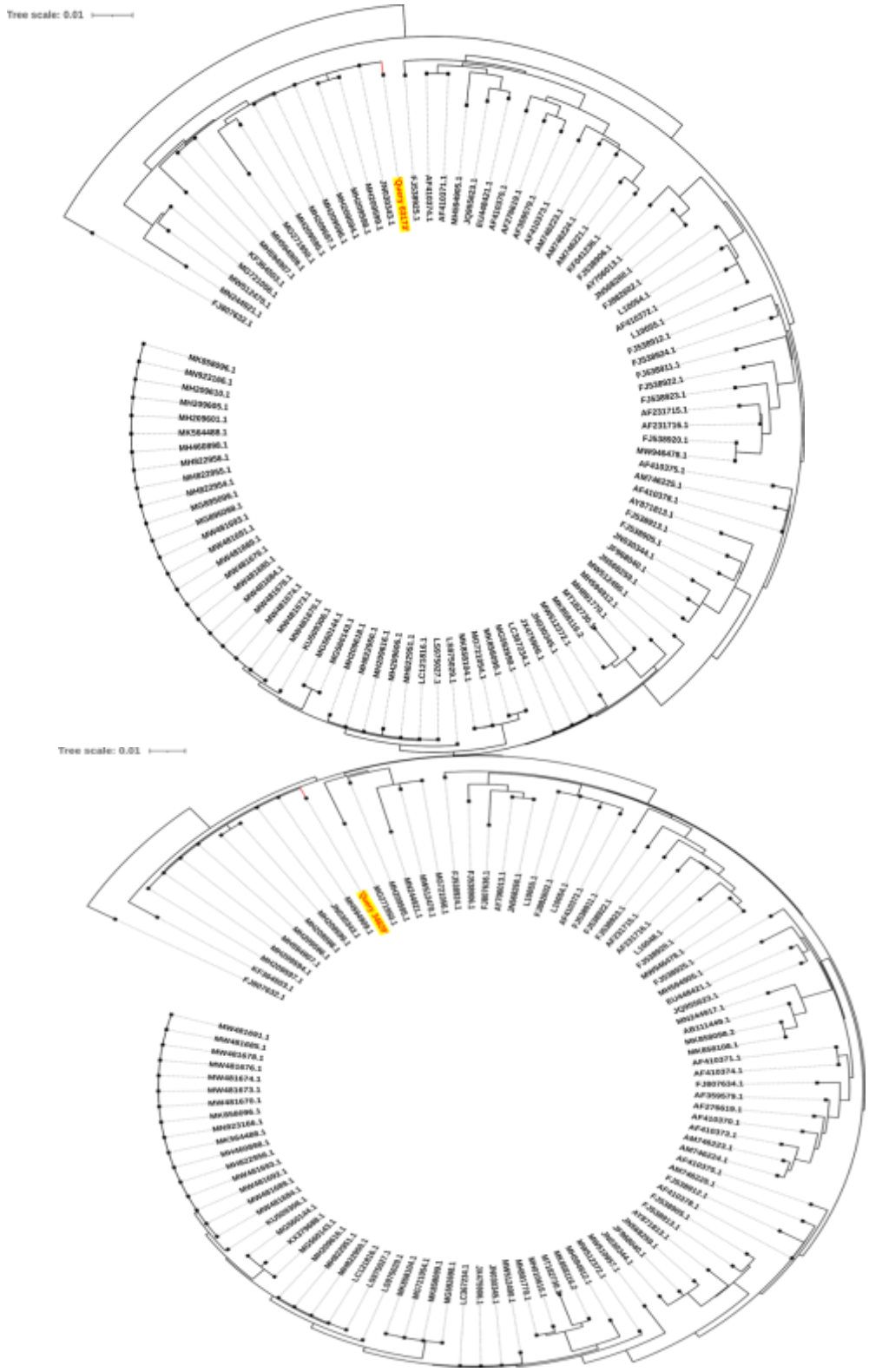

Figure 3: Phylogenetic tree of DENV-2. (a) DF-1 and (b) DR-1The maximum-likelihood tree was constructed based on the complete envelope gene sequences generated during this study and retrieved from