Advances in Bioresearch
Adv. Biores., Vol 15 (6) November 2024: 395-404
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.6.395404

Advances
in
Bioresearch

ORIGINAL ARTICLE

Comparative Study of Sequence & Phylogeny Analysis of Dengue Virus isolates from globally infected countries

Debojyoti Dutta¹, Semanti Ghosh¹
1Department of Biotechnology, Swami Vivekananda University, Barrackpore, Kolkata – 700121, India.
*Corresponding Author's Email: semantig@svu.ac.in

ABSTRACT

Dengue is a illness consist of four serotypes (DENV 1 to 4). The dengue virus isolates also seen as there genotype i.e., 1, 2, 3 and many more. Due to dengue virus disease is still untreated we decided to analyze the gene sequence of dengue virus from different regions of the globe, like Africa, Asia, North America, Oceania and South America by different bioinformatics software and tools. Our research presents an extensive comparative analysis between the circulating Dengue virus serotypes in global through both sequence and evolutionary analyses. The data set included Dengue virus sequences collected from various countries Africa, Asia, North America, Oceania, South America obtained from the NCBI Virus database. We performed sequence alignment along with phylogeny analyses using MEGA11 to examine genetic diversity as well as evolutionary relationships among these serotypes. Results revealed distinct clustering patterns among Dengue virus serotypes with their genotypes indicating multiple lineages present. Phylogeny analysis suggests local evolution alongside strains introduced from neighboring regions and protein identification for drug treatment. Multiple sequence alignment, phylogenetic tree and protein generation from query nucleotide sequence can highlight the comparison behind other sequences and identifying target proteins. Sequence analysis for identification of differentiation or, similarity of strains of same serotypes. Exploring the evolutionary relationships between isolates through phylogenetic analysis highlighted on the dynamic nature of DENV evolution, highlighting patterns of divergence and common ancestry. The study also identified proteins from the target genome sequence for potential drug treatment.

Keywords: *Dengue Virus, Genotype, Multiple Sequence Alignment, Nucleotide, Phylogenetic Tree, Protein, Serotype*

Received 04.10.2024 Revised 21.10.2024 Accepted 23.11.2024

How to cite this article:
Mouli N, Ananya S, Semanti G. Identification of Key Genes Related to Salinity Stress in Rice (*Oryza sativa*) Using Bioinformatics Analysis. Adv. Biores., Vol 15 (6) November 2024: 395-404.

INTRODUCTION

Dengue fever was caused by the Dengue virus (DENV) in the past published reports from WHO (World Health Organization). A single stranded positive RNA (ribonucleic acid) virus, belonging to the genus *Flavivirus* in the family *Flaviviridae*, was transmitted by Aedes mosquitoes [1, 2]. The fifth reported serotype was not confirmed, while four serotypes had been found [3, 4, 5]. In 1984, DENV-1 (Dengue virus serotype 1) was reported to be active in Africa, indicating a wide distribution of this serotypes [6]. DENV-2 (Dengue virus serotype 2) split from its ancestors in the Sylvatic region approximately 400 to 600 years ago. This difference represented an important evolutionary step the virus took in adapting to its human host. The first reports of DENV-2 (Dengue virus serotype 2) occurred in 1944 in Asia. Reports from Nigeria, Africa in 1964 demonstrated that the virus could affect diverse populations around the world after this. In 1953, DENV-2 (Dengue virus serotype 2) was recorded in North and South America, as well as in the Republic of Trinidad and Tobago, demonstrating the wide geographical distribution of the serotypes [6]. DENV-3 (Dengue virus serotype 3) had been first recorded in 1953 in Asia. Reports came from the Philippines and Thailand. In 1963, this initial discovery of DENV-3 (Dengue virus serotype 3) was reported in Puerto Rico, North America. Subsequently, it was also reported in Mozambique, Africa, between 1984 and 1985. DENV-4 (Dengue virus serotype 4) was first reported in 1953 in Asia. The initial discoveries were made in the Philippines and Thailand. DENV-4 (Dengue virus serotype 4) was reported in the North and South America, with Brazil, Cuba, Dominica, Puerto Rico, and the US Virgin Islands noting its presence in 1981 in the past. Five distinct genotypes of DENV-1 (Dengue virus serotype 1) were identified based on complete E (Envelope) gene sequences [7], E/NS1 (Envelope/Nonstructural protein 1) gene junction [8], Five genotypes were defined for DENV-1 (Dengue virus serotype 1). Six were identified for DENV-2 (Dengue virus serotype 2). Five were determined for DENV-3 (Dengue virus serotype 3). Four were identified for DENV-4 (Dengue virus serotype 4). In the past, DENV-2 (Dengue virus serotype 2) was divided into six genotypes: Asian I, Asian II, American, Asian/American, Wild, and Cosmopolitan [9, 10, 11]. In this study we conducted multiple sequence alignment, phylogeny analysis of 38 whole nucleotide sequences of four genotypes of Dengue Virus isolated from different countries. Further, we build 3D model of translated protein sequence of indian dengue virus type 3 serotype. This study will show the sequence differentiation in genotypes of diverse Dengue Viruses and future prospect of therapeutic target prediction on the basis of protein model.

MATERIAL AND METHODS

Isolation Of Dengue Virus Genes

Blood samples of Dengue virus, Dengue virus type 1, type 2, type 3, type 4, and dengue virus type I obtained along with their genotypes, from various dengue-infected countries like Africa (Figure 1), Asia (Figure 2), North America & South America (Figure 3) and Oceania (Figure 4). The information about the (+) ssRNA (positive single stranded ribonucleic acid) sequence, which was a nucleotide sequence, was retrieved from Virus database by researchers from various organizations [12] in NCBI (National Center for Biotechnology Information) [13]. The complete nucleotide sequences of the dengue virus isolate, along with their accession numbers, were obtained from the GenBank database [14] in NCBI (National Center for Biotechnology Information) [13] (Table 1).

Multiple Sequence Alignment (MSA)

The MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm was used to aligned all 38 nucleotide sequences [15] via MEGA 11 (Molecular Evolutionary Genetics Analysis version 11) software [16] and visualized using BioEdit (biological sequence alignment editor) software [17]. The BioEdit (Biological sequence alignment Editor) software was used to visualize the MSA (multiple sequence alignment), which was generated using MEGA 11 (Molecular Evolutionary Genetics Analysis version 11) [16] and the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm [15]. Using default parameters, gap penalties (open distance = -400) were applied and UPGMA (unweighted pair group method with arithmetic mean) cluster method was employed for aligning nucleotides [18].

Phylogenetic Tree Analysis

MEGA 11 (Molecular Evolutionary Genetics Analysis version 11) software was used to perform and visualize the phylogenetic analysis [16]. The Neighbor-Joining method was used to infer the evolutionary history [19]. In the bootstrap test, 500 percent of the replicate trees had the associated taxa clustering together [20]. Evolutionary analyses were conducted in MEGA 11 (Molecular Evolutionary Genetics Analysis version 11) [16].

Proteins Retrieved from Whole Genome Sequences

Through a multi-step bioinformatics process, genome sequences yield the various coding sequences along with its translated proteins. The Coding Deoxyribonucleic Acid Sequences (CDS) were translated into protein sequences based on the genetic code, and the resulting mature peptides (mat_peptide) were deposited in the GenBank database [14] in NCBI (National Center for Biotechnology Information) [13]. The genome (nucleotide) sequence of the individual isolate produces the gene, which in turn generates as structural and non-structural proteins of dengue virus (Table 2) in the different countries. Here the query sequence, i.e., Indian dengue virus type 3 sequence (Accession no. GQ466079.1) compared to other 38 dengue sequences were found longer than many other sequences. To find out the corresponding protein sequence of this nucleotide sequence (Accession no. GQ466079.1), the protein BLAST was used (Basic Local Alignment Search Tool) [21] against PDB (The Protein Data Bank) repository [22]. Further, the protein three-dimensional model was generated from the obtained protein sequence (Accession no. ADM63678.1) by homology model method using SWISS-MODEL [23] and then the proteins were also validated and visualized.

RESULT AND DISCUSSION

Alignment Of Dengue Virus Serotypes

In MSA (multiple sequence alignment), the followed full sequences of the mentioned dengue virus strains/isolates like dengue virus, dengue virus type 1, type 2, type 3, type 4 and dengue virus type I were generated in Bitmap image (Supplementary Figure 1). All ambiguous positions were removed for each sequence pair (pairwise deletion option). The shading of the identical and similarity positions of nucleotide were generated by using BLOSUM62 (Blocks Substitution Matrix 62) [24]. Here, in sequence alignment the mostly conserved as well as similar and identical positions of nucleotides were seen from 52 position to 10,680 positions of query sequence of India dengue virus isolates.

Phylogenetic analysis of Dengue Virus serotypes

In the phylogeny the optimal tree was shown in Figure 5 and the serotypes of the dengue virus were marked as their respected nucleotide sequence accession number, country name and genotypes like genotype 1, genotype 2, genotype 3, genotype 4, genotype cosmopolitan, genotype dengue 2, genotype dengue 3, genotype dengue 4, genotype DENV-2 (dengue virus serotype 2). The evolutionary distances were computed using the Kimura 2-parameter method [25] and were in the units of the number of base substitutions per site. This analysis involved 38 nucleotide sequences. Each genotype included multiple strains from different geographical locations. In this phylogenetic tree analysis (Figure 5), we seen that here, tree clusters were mentioned by their genotypes of dengue virus sequences. In the phylogeny we observed that genotype 1 cluster was a biggest cluster followed by genotype 2, genotype 4, genotype 3 and smallest clusters were genotype Dengue 2, genotype Dengue 3, genotype Dengue 4, genotype DENV-2 and genotype Cosmopolitan. Here, in genotype 1 cluster Cambodia sequence and Laos sequence were connected to same cluster, Myanmar sequence and Sri Lanka sequence were connected to another cluster and also, we observed that this clusters were connected with same node. Here Eritrea sequence was in separate node. French Polynesia sequence and USA sequence was connected in closest node, Chile sequence and Indonesia sequence were in separate nodes. Here Indonesia sequence's node length was greater than Chile sequence's node. Argentina sequence and Jamaica sequence was connected to same cluster, Bangladesh sequence and South Korea sequence was connected to other cluster and these two clusters were connected in same node. In genotype 3 cluster Australia sequence and Philippines sequence was connected to same cluster and Paraguay sequence was in separated node, genotype 3 cluster's Gabon sequence and genotype Dengue 3 cluster's India sequence was connected in same node. In genotype 2 cluster Malaysia sequence was in separated node, Fiji and New Caledonia sequences were connected to same cluster and Tonga sequence was in separated node. Guatemala sequence and Peru sequence of genotype 2 cluster was connected in same cluster. Brunei sequence of genotype 2 cluster and Singapore sequence of genotype DENV-2 cluster was connected to same cluster, Vietnam sequence of genotype cosmopolitan cluster and Pakistan sequence of genotype 2 cluster was in separated nodes. Thailand sequence of genotype Dengue 2 cluster was in separated node, Taiwan sequence of genotype 2 cluster was in separated node, Mexico sequence and Papua New Guinea sequence of genotype 2 cluster was connected in same node. Here Taiwan sequence, Mexico sequence, Papua New Guinea sequence was in genotype 2 cluster. Senegal sequence of genotype 4 and Columbia sequence of genotype Dengue 4 was connected in same cluster, Venezuela sequence, Haiti sequence, Brazil sequence and China sequence of genotype 4 cluster was in separated nodes. Here we also observed that Gabon strain, Paraguay strain and Indian dengue strain were forming cluster and they were identical with each other. In phylogeny study we seen that, India sequence was in type 3 serotype of Dengue virus but it was different in its genotype comparing to other type 3 serotype sequences of other countries.

Protein identification from complete nucleotide sequences

The protein sequence information about various dengue virus types from countries such as South Korea, Taiwan, Venezuela, Vietnam, Mexico, Myanmar, and others., including their associated proteins and genome sequences were tabulated in supplementary Table 1. It provided details about the presence of specific proteins such as capsid protein (C), membrane glycoprotein precursor, envelope protein (E), nonstructural proteins 1 (NS 1), nonstructural proteins 2A (NS 2A), nonstructural proteins 2B (NS 2B), nonstructural proteins 3 (NS 3), nonstructural proteins 4A (NS 4A), nonstructural proteins 4B (NS 4B), nonstructural proteins 5 (NS 5) and other proteins like (prM) precursor membrane protein. The genetic sequences and polyproteins of the dengue virus types were also mentioned, along with their complete full lengths and 38 countries, where this dengue strains were isolated. The certain proteins like 2K (2 kinase) protein, NS4A (nonstructural protein 4a), NS4B (nonstructural protein 4b), ribonucleic acid dependent ribonucleic acid polymerase (RdRp) and NS5 (nonstructural protein 5) was found that are translated from their respective nucleotide sequences. In this case we collected the protein sequence (protein accession: ADM63678.1 collected from Indian dengue virus nucleotide sequence) of the India from the GenBank database [14] in NCBI (National Center for Biotechnology Information) [13]. Here dengue virus sequence of India was selected because, in phylogenetic tree analysis of serotype Dengue 3 we observed that except Australia sequence, the other sequences from Philippines, Paraguay, Gabon and India sequence were in closely identical and neighbourly joined but their respective proteins or peptides were not reported. The India sequence of genotype 3 further taken for homology modelling after the protein template search in SWISS-MODEL [23]. In structure assessment of protein model from 8gzr template, the protein model's coverage region was 2496-3375 and we validated in ramachandran plot (Figure 6 A-B).

During structure validation in Swiss Model we found that MolProbity score was 0.81, Clash Score 0.28, Ramachandran Favoured value was 96.70%, Rotamer Outliers was 0.11%, C-Beta Deviations was 2, Bad Bonds was 0 out of 7300, Bad Angles was 25 out of 9877. Here, built 3D model of protein had 23 alpha-helices and 4 beta-sheets. The secondary structural details of the protein were presented in Table 2. Here in this study, the protein model will help for drug interaction against dengue protein and for future therapeutic purposes.

Table 1: List of Complete Nucleotide Sequence data.

Accession	Organism Name	Org_location	Release_Date	Species	Molecule type	Length	Genotype	Country	Host	Isolation Source	Collection Date
DQ672560.1	dengue virus type I	USA	01-07-2007	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	French Polynesia	Homo sapiens	blood	2001
DQ672561.1	dengue virus type I	USA	01-07-2007	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	USA	Homo sapiens	blood	2001
EU179857.1	Dengue virus type 2	Malaysia	14-10-2007	*Orthoflavivirus denguei*	ssRNA(+)	10709	2	Brunei	Homo sapiens	blood	2005
EU863650.1	dengue virus type I	Chile	20-10-2008	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	Chile	Homo sapiens	blood	2002
FJ467493.1	Dengue virus type 2	Malaysia	15-05-2009	*Orthoflavivirus denguei*	ssRNA(+)	10719	2	Malaysia	Homo sapiens	blood	2008
GQ466079.1 (Query)	Dengue virus type 3	India	02-05-2011	*Orthoflavivirus denguei*	ssRNA(+)	10680	Dengue 3	India	Homo sapiens	blood	2008
GU289913.1	Dengue virus type 4	USA	17-12-2009	*Orthoflavivirus denguei*	ssRNA(+)	10649	dengue 4	Colombia	Homo sapiens	blood	1982
GU289914.1	Dengue virus type 2	USA	18-12-2009	*Orthoflavivirus denguei*	ssRNA(+)	10723	dengue 2	Thailand	Homo sapiens	blood	1974
HM582099.1	Dengue virus type 2	USA	25-08-2010	*Orthoflavivirus denguei*	ssRNA(+)	10714	2	Fiji	Homo sapiens	blood	1971
HM582102.1	Dengue virus type 2	USA	25-08-2010	*Orthoflavivirus denguei*	ssRNA(+)	10713	2	New Caledonia	Homo sapiens	blood	1971
HM582111.1	Dengue virus type 2	USA	25-08-2010	*Orthoflavivirus denguei*	ssRNA(+)	10713	2	Tonga	Homo sapiens	blood	1974
HQ235027.1	Dengue virus type 3	Brazil	28-05-2011	*Orthoflavivirus denguei*	ssRNA(+)	10707	3	Paraguay	Homo sapiens	blood	2007
HQ999999.1	Dengue virus type 2	USA	06-11-2011	*Orthoflavivirus denguei*	ssRNA(+)	10725	2	Guatemala	Homo sapiens	blood	2009
JF262781.1	Dengue virus type 4	USA	08-01-2012	*Orthoflavivirus denguei*	ssRNA(+)	10649	4	Venezuela	Homo sapiens	blood	1995
JF262782.1	Dengue virus type 4	USA	08-01-2012	*Orthoflavivirus denguei*	ssRNA(+)	10649	4	Haiti	Homo sapiens	blood	1994
JF327392.1	Dengue virus type 2	Singapore	21-06-2011	*Orthoflavivirus denguei*	ssRNA(+)	10723	DENV-2	Singapore	Homo sapiens	blood	2009
JN054255.1	dengue virus type I	Sri Lanka	23-07-2011	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	Sri Lanka	Homo sapiens	blood	2010

JN406514.1	Dengue virus type 3	Australia	01-08-2012	*Orthoflavivirus denguei*	ssRNA(+)	10707	3	Australia	Homo sapiens	blood	1998
JN983813.1	Dengue virus type 4	Brazil	22-01-2012	*Orthoflavivirus denguei*	ssRNA(+)	10649	4	Brazil	Homo sapiens	blood	08-09-2010
JQ822247.1	Dengue virus type 4	China	05-06-2012	*Orthoflavivirus denguei*	ssRNA(+)	10653	4	China	Homo sapiens	blood	2010
KC294200.1	Dengue virus type 2	Peru	10-01-2014	*Orthoflavivirus denguei*	ssRNA(+)	10724	2	Peru	Homo sapiens	blood	28-03-2007
KC692495.1	dengue virus type I	Argentina	12-08-2014	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	Argentina	Homo sapiens	blood	03-02-2009
KC762620.1	Dengue virus 1	Indonesia	17-01-2015	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	Indonesia	Homo sapiens	blood	13-12-2007
KF041232.1	Dengue virus type 2	Singapore	23-12-2013	*Orthoflavivirus denguei*	ssRNA(+)	10723	2	Pakistan	Homo sapiens	blood	2011
KF907503.1	Dengue virus	France	01-03-2014	*Orthoflavivirus denguei*	ssRNA(+)	10648	4	Senegal	Homo sapiens	blood	1953
KJ734727.1	Dengue virus type 2	Taiwan	19-05-2014	*Orthoflavivirus denguei*	ssRNA(+)	10723	2	Taiwan	Homo sapiens	blood	2014
KM204118.1	Dengue virus type 2	USA	01-11-2014	*Orthoflavivirus denguei*	ssRNA(+)	10723	2	Papua New Guinea	Homo sapiens	blood	1944
KP406801.1	dengue virus type I	South Korea	12-10-2015	*Orthoflavivirus denguei*	ssRNA(+)	10736	1	South Korea	Homo sapiens	blood	2004
KU050695.1	Dengue virus type 3	USA	15-12-2015	*Orthoflavivirus denguei*	ssRNA(+)	10696	3	Philippines	Homo sapiens	blood	1956
KU509249.1	dengue virus type I	Germany	25-01-2017	*Orthoflavivirus denguei*	ssRNA(+)	10582	1	Jamaica	Homo sapiens	blood	2012
KU509258.1	dengue virus type I	Germany	25-01-2017	*Orthoflavivirus denguei*	ssRNA(+)	10377	1	Eritrea	Homo sapiens	blood	2010
KU509260.1	dengue virus type I	Germany	25-01-2017	*Orthoflavivirus denguei*	ssRNA(+)	10378	1	Cambodia	Homo sapiens	blood	2008
LC379193.1	Dengue virus type 3	Japan	12-12-2019	*Orthoflavivirus denguei*	ssRNA(+)	10663	3	Gabon	Homo sapiens	blood	15-05-2016
LC436667.1	dengue virus type I	Thailand	03-06-2020	*Orthoflavivirus denguei*	ssRNA(+)	10673	1	Bangladesh	Homo sapiens	blood	2017-10
MG679800.1	dengue virus type I	China	03-08-2018	*Orthoflavivirus denguei*	ssRNA(+)	10735	1	Myanmar	Homo sapiens	blood	27-10-2017
MG877552.1	dengue virus type I	France	04-09-2018	*Orthoflavivirus denguei*	ssRNA(+)	10625	1	Laos	Homo sapiens	blood	2012
MH613984.1	Dengue virus type 2	USA	13-03-2019	*Orthoflavivirus denguei*	ssRNA(+)	10723	2	Mexico	Aedes albopictus	blood	2017-12
OQ832616.1	dengue virus type 2	Japan	20-06-2023	*O. denguei*	ssRNA(+)	10723	Cosmopolitan	Viet Nam	Homo sapiens	blood	2019-10

Table 2: Secondary structural details of 8gzr protein.

Secondary structure	Amino acid residues of 8gzr
Alpha-Helix	GLU2501-LYS2519, GLU2530-LYS2536, TYR2579-CYS2581, GLU2647-GLU2659, GLU2681-HIS2690, GLU2768-GLU2776, LYS2815-ASP2822, ARG2842-GLU2846, ARG2860-ARG2872, ARG2885-LYS2891, GLU2918-LYS2932, ARG2971-GLU2983, HIS3002-ASP3010, ASP3028-LYS3041, GLU3049-LYS3059, TYR3096-ASP3122, GLU3130-LYS3146, ASP3162-ASP3173, ASP3222-ARG3227, CYS3243-TYR3248, ARG3260-CYS3270, GLU3297-ARG3305, LYS3331-ASP3334
Beta-Sheet	GLU2589-TYR2593, TYR2794-GLU2799, LYS3065-ARG3084, HIS3202-GLU3205

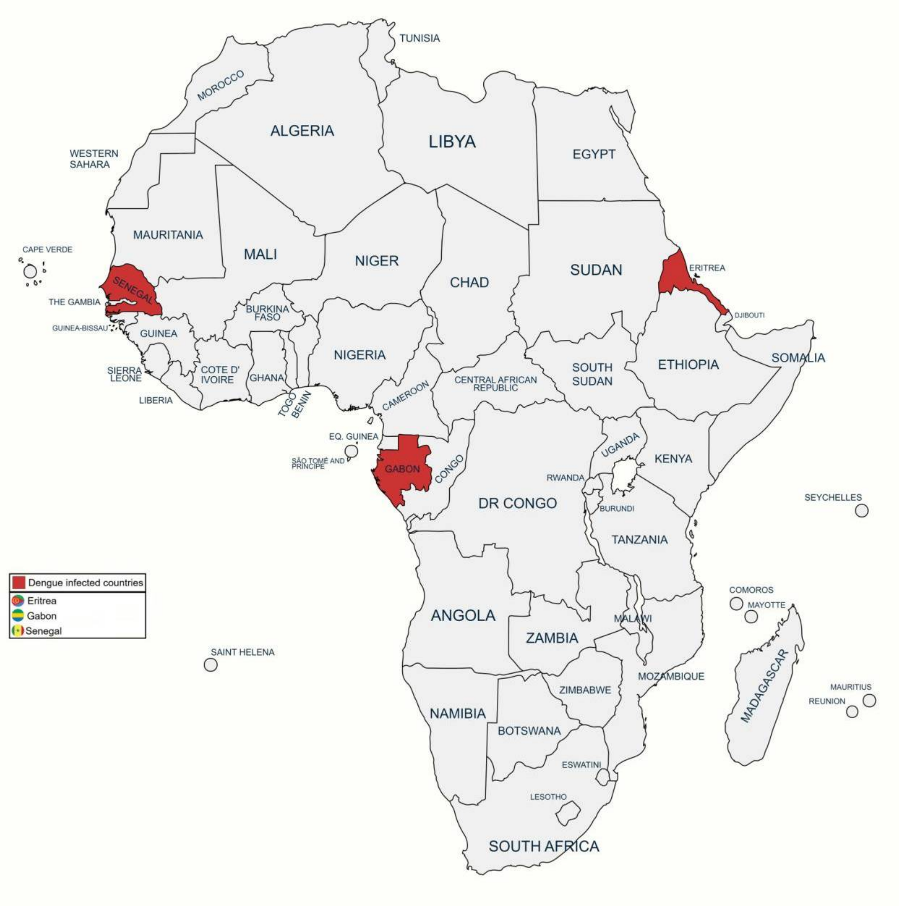

Figure 1: Dengue infected countries in Africa.

Figure 2: Dengue infected countries in Asia.

Figure 3: Dengue infected countries in North & South America.

Figure 4: Dengue infected countries in Oceania.

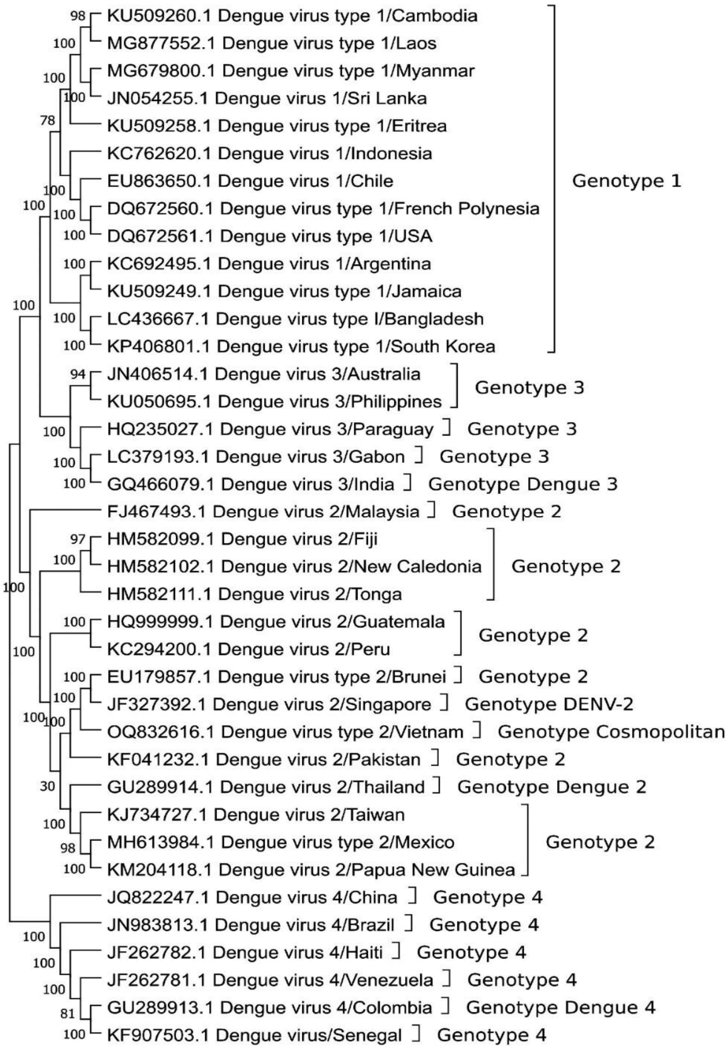

Figure 5: Phylogeny of 38 isolates.

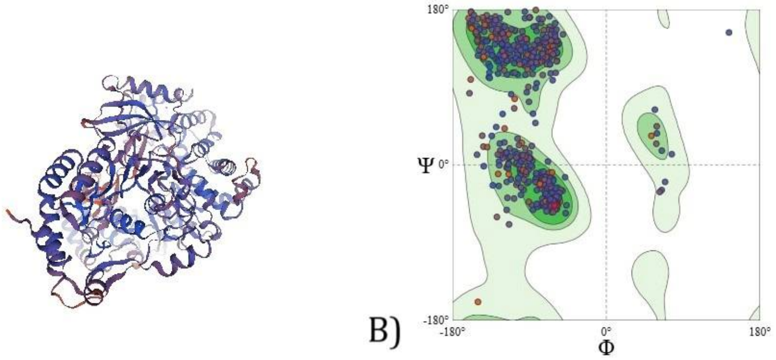

Figure 6: A) Tertiary structure of protein model, B) Ramachandran plot of modelled protein

CONCLUSION

In conclusion, the comparative study of sequence and phylogeny analysis of Dengue virus isolates from globally infected countries revealed distinctive clustering patterns among Dengue virus serotypes and their genotypes, indicating the presence of multiple lineages. The phylogenetic analysis highlighted the dynamic nature of DENV (Dengue virus) evolution, shedding light on patterns of divergence and common ancestry. This analysis provided valuable insights into the genetic relationships between different types of Dengue viruses present in the global evolutionary landscape, emphasizing the significance of understanding the evolutionary dynamics of these viruses in a global context. Furthermore, the sequence analysis allowed for the identification of differentiation or similarity of strains of the same serotypes, offering crucial information on the genetic and functional diversity present in five continents, including India. The study also identified proteins from the target genome sequence for potential drug finding, enhancing our understanding of the genetic makeup of the virus and providing potential targets for future research and therapeutic interventions. Overall, this research contributes to the ongoing efforts to comprehend the complex dynamics of Dengue virus evolution and the global spread of the virus. By analyzing sequences from diverse geographic regions, this study has provided valuable insights that could inform the development of strategies for the prevention and control of Dengue virus, contributing to the advancement of public health initiatives worldwide.

Conflict of Interest

None

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