Egypt. J. Biophys. Biomed. Eng., **Vol. 20,** pp 29- 36 (2019)

Ebola Virus L Polymerase RdRp Sequence and Phylogenetic Analysis

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EBOLA Virus (EBOV) infection affects humans beings in the last four decades with the deadliest outbreak at 2015 in West Africa, leaving more than 10,000 deaths. The virus harms the liver of the patient when direct contact with contaminated body fluids or blood is occurred. L polymerase is one of the viral proteins responsible for the viral RNA replication. Inhibition of the viral polymerase succeeded is in stopping the infection of other viruses such as Hepatitis C Virus (HCV). National Center for Biotechnology Information (NCBI) protein database has 2123 sequences for L polymerase. In present research, the sequence and phylogenetic analysis are utilized to understand the non-redundant sequence coming from different countries. Based on the sequence similarity, the solved structure of vesicular stomatitis virus (PDB ID: 5A22) is used in this work to suggest the active site of the EBOV RdRp domain. Two newly released sequences (APT69557.1 and ALX33626.1 for the Sudan and Zaire, respectively) are based on the phylogenetic analysis, show interestingly a divert, mutation and distance from its subgroups suggesting a new emerged isoform of EBOV RdRp. The active site motif, GDN, would be targeted by polymerase inhibitors succeeded in other viruses to get stop the infection.

Keywords: L polymerase, EBOV, GDN motif, phylogenetic analysis, sequence alignment.

Introduction

Ebola virus is stand out amongst the most harmful pathogens known to contaminate people.[1] The first recognized Ebola outbreak occurred at 1976, near Ebola River in Zaire (now Democratic Republic of Congo, DRC). In recent years, more than 20 flare-ups have happened in Africa, with the vast majority of the known episodes happenned in the previous 20 years.[2]

The main routes of Ebola virus transmission are direct contact with asymptomatic Ebola patient's blood and body fluids (including but not restricted to urine, feces, vomitus, saliva, and sweat) through breaks in the skin or inoculation into the mouth, nose or eyes. Human contamination can likewise happen through contact with wild animals, such as by hunting, butchering or preparing meat from infected animals[3]. Ebolavirus (EBOV) causes an exceptionally infectious zoonotic disease, affects humans and other primates. Although the natural outbreak of the EBOV is yet restricted to Africa, fast methods for individual's correspondence, high viral transmissibility, and high mortality rate have made the EBOV a serious global health threat. Currently, there is no effective direct acting anti-EBOV drug. EBOV patients receive only palliative therapy.[4]

Currently, there are no licensed vaccines or treatments available to combat EBOV disease and as such, research aimed at identifying targets for therapeutic intervention is of high priority. However, the classification of EBOV as a biosafety level 4 (BSL-4) pathogen greatly limits studies using a live virus.[5] Nearly every Ebola virus protein has been characterized for therapeutic targeting potential.[6]

The EBOV genome is a negative-sense single-stranded RNA and contains a viral envelope, matrix, and nucleocapsid components. It encodes seven structural proteins: nucleoprotein (NP), polymerase cofactor (VP35, VP40, GP), transcription activator (VP30, VP24), and RNA-dependent RNA polymerase (L).[7,8] Viral RNA-dependent RNA polymerases (RdRp) are essential for replication of RNA viruses and represent important drug targets.[9]

CLUSTALΩ [10] is web -based software service for performing fast and accurate multiple sequence alignments (MSAs) of potentially large

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DOI :10.21608/ejbbe.2019.17450.1027

numbers of protein or DNA/RNA sequences. It is the latest version of the famous and widely used CLUSTAL MSAs . [11,12].

The aim of present work to determine the active site of RdRp of EBOV to use in docking method with anti-viral drugs. Study the relationship of EBOV L polymerase sequence by pairwise distance and phylogenetic tree.

Materials and Methods

Multiple sequence alignment

Multiple sequence alignment was performed using the web server CLUSTALΩ [10]. The alignment was ordered and represented using Easy Sequencing in PostScript (ESPript 3.0) web server[13,14]. ESPript 3.0 generates figures of aligned sequences with secondary structure information. It can serve as a tool for structure/ function analyses. ESPript reads text outputs from multiple sequence alignment programs such as CLUSTALΩ and MULTALIN, as well as from programs able to identify secondary structure elements from structure files such as DSSP[15] and STRIDE[16].

EBOV polymerase Sequence analysis

2123 of EBOV L polymerase protein representing all recorded EBOV outbreaks as retrieved from the National Center for Biotechnology Information (NCBI) http:// www.ncbi.nlm.nih.gov/,[17]. EBOV L polymerase Sequence were downloaded from various countries (Sudan, Reston, Bombali, Zaire, Tai Forest and Bundibugyo) and synthetic construct (that Artificial viruses to understand and prevent viral disease).

EBOV polymerase Sequence selected

21 unique sequences for EBOV L polymerase protein were selsected. Eight from Sudan, three from Reston (United States), two from Bombali (Sierra Leone), three from Zaire, two from Tai Forest (Côte d'Ivoire), two from Bundibugyo (Uganda) and one synthetic construct.

Pairwise distance method and (MEGA) software

Pairwise distance method is used to test the distances between the aligned sequences using Molecular Evolutional Genetics Analysis (MEGA) software [18] in table 1.

Phylogenetic tree

Phylogenetic tree of the aligned sequences is also calculated using MEGA software and represented by the Cladogram.

Results and Discussion

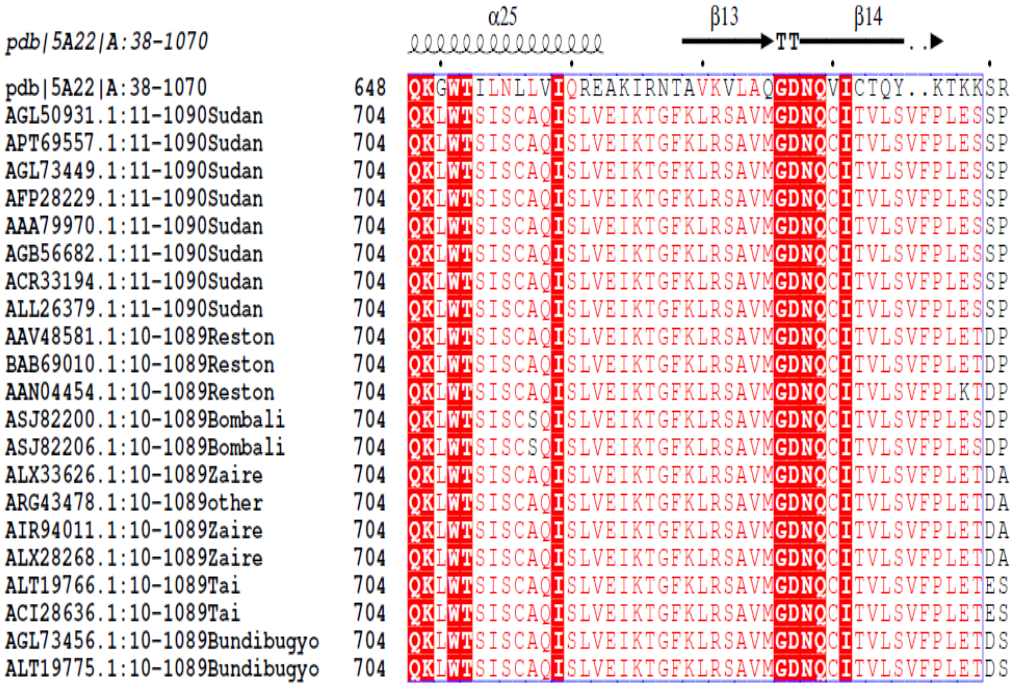
Sequence alignment

To obtain the correct alignment of a large set of sequences, with some of them being only distantly related, it is essential to elaborate an optimal scheme for hierarchical alignment and to delimit the portions of the sequences which are optimal for revealing non-random similarity. Sequences of various sets of negative-strand viral RNA of Ebola polymerases have been downloaded from NCBI http://www.ncbi.nlm.nih.gov/,[17]., The authors used CLUSTALΩ website [10] to perform multiple sequence alignment of the 21 sequences of RdRp of EBOV L polymerase. The total number of downloaded sequences are 2123 according to various countries (Sudan, Reston, Bombali, Zaire, Tai Forest, Bundibugyo) and synthetic construct.

After reducing the sequences for the RdRp domain, the number of non-redundant sequences becomes 21. The alignment is represented by ESPript 3.0 as shown in Fig.1. The alignment consists of 1082 amino acids and includes distinct blocks of amino acid residues which could be considered conserved motifs. Overall the sequence similarity is high (highlighted in red in Fig.1). The highest sequence identity is 99.91%, and the lowest is 80.74%. The secondary structure of the cryo-EM solved L protein of vesicular stomatitis virus (PDB ID: 5A22) is represented in the top of the alignment. This solved structure represents the best homolog for building the 3D model of EBOV RdRp (89 % coverage and sequence Identity 17.4%). The sequence of the solved structure (PDB ID: 5A22) is aligned against EBOV sequences. Based on the multiple sequence alignment, GDN motif (the reported active site of L protein of vesicular stomatitis virus) is conserved in EBOV with the two aspartate residues protruding from the beta-turn structure (between β13 and β14). This is suggested to be the active site for EBOV RdRp.

Pairwise distance analysis

The pairwise distance for the aligned sequences is represented in table 1. Pairwise distance, that is the minimum number of changes necessary to convert one sequence into another, for the sequences from the same country show shorter values, while the distances increase when aligning two sequences from different countries. Interestingly, Sudan EBOV shows the shortest distances compared to other EBOV sequence under the study. This implies the phylogenetic relevance as reported from the phylogenetic tree shown in Fig. 2.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β13
β14
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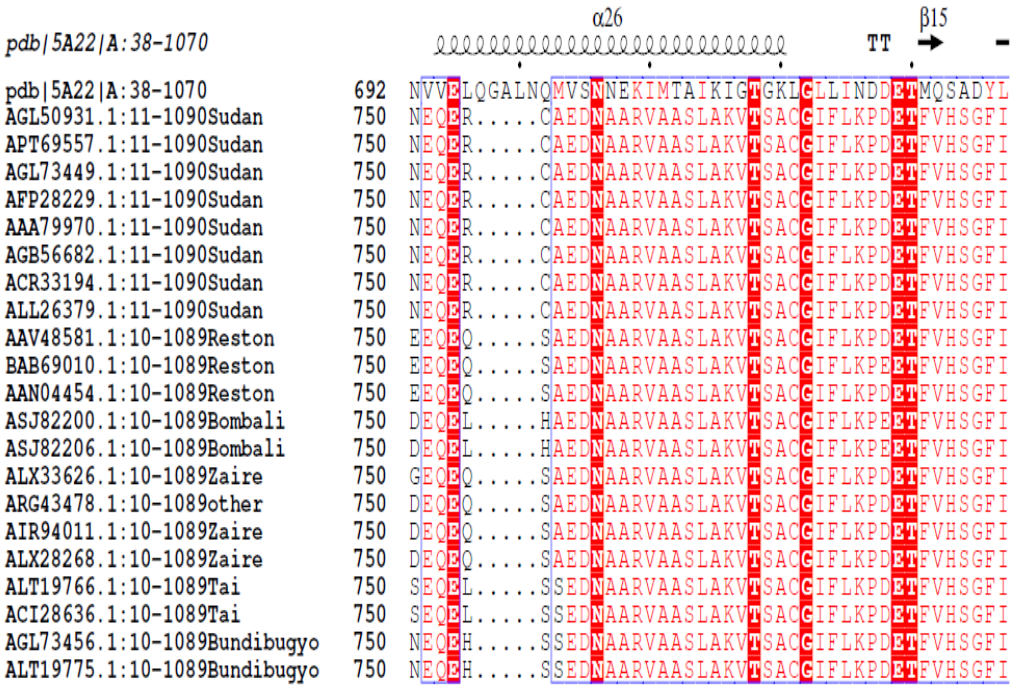
pdb|5A22|A:38-1070 648 QKGWTILNLLVIQREAKIRNTAVKVLAQGDNQVICTQY..KTKKSR
AGL50931.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
APT69557.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
AGL73449.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
AFP28229.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
AAA79970.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
AGB56682.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
ACR33194.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
ALL26379.1:11-1090Sudan 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESSP
AAV48581.1:10-1089Reston 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDP
BAB69010.1:10-1089Reston 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDP
AAN04454.1:10-1089Reston 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLKTDP
ASJ82200.1:10-1089Bombali 704 QKLWTSISCSQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESDP
ASJ82206.1:10-1089Bombali 704 QKLWTSISCSQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLESDP
ALX33626.1:10-1089Zaire 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDA
ARG43478.1:10-1089other 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDA
AIR94011.1:10-1089Zaire 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDA
ALX28268.1:10-1089Zaire 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDA
ALT19766.1:10-1089Tai 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETES
ACI28636.1:10-1089Tai 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETES
AGL73456.1:10-1089Bundibugyo 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDS
ALT19775.1:10-1089Bundibugyo 704 QKLWTSISCAQISLVEIKTGFKLRSAVMGDNQCITVLSVFPLETDS


α26
β15
pdb|5A22|A:38-1070
pdb|5A22|A:38-1070 692 NVVELQGALNQMVSNNEKIMTAIKIGTGKLGLLINDDETMQSADYL
AGL50931.1:11-1090Sudan 750 NEQERCAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
APT69557.1:11-1090Sudan 750 NEQERCAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
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AGB56682.1:11-1090Sudan 750 NEQERCAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
ACR33194.1:11-1090Sudan 750 NEQERCAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
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AAV48581.1:10-1089Reston 750 EEQEQSAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
BAB69010.1:10-1089Reston 750 EEQEQSAEDNAARVAASLAKVTSAGGIFLKPEETFVHSGFI
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ASJ82200.1:10-1089Bombali 750 DEQELHAEDNAARVAASLAKVTSAGGIFLKPEETFVHSGFI
ASJ82206.1:10-1089Bombali 750 DEQELHAEDNAARVAASLAKVTSAGGIFLKPEETFVHSGFI
ALX33626.1:10-1089Zaire 750 GEQEQSAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
ARG43478.1:10-1089other 750 DEQEQSAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
AIR94011.1:10-1089Zaire 750 DEQEQSAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
ALX28268.1:10-1089Zaire 750 DEQEQSAEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
ALT19766.1:10-1089Tai 750 SEQELSSEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
ACI28636.1:10-1089Tai 750 SEQELSSEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
AGL73456.1:10-1089Bundibugyo 750 NEQEHSSEDNAARVAASLAKVTSAGGIFLKPDETFVHSGFI
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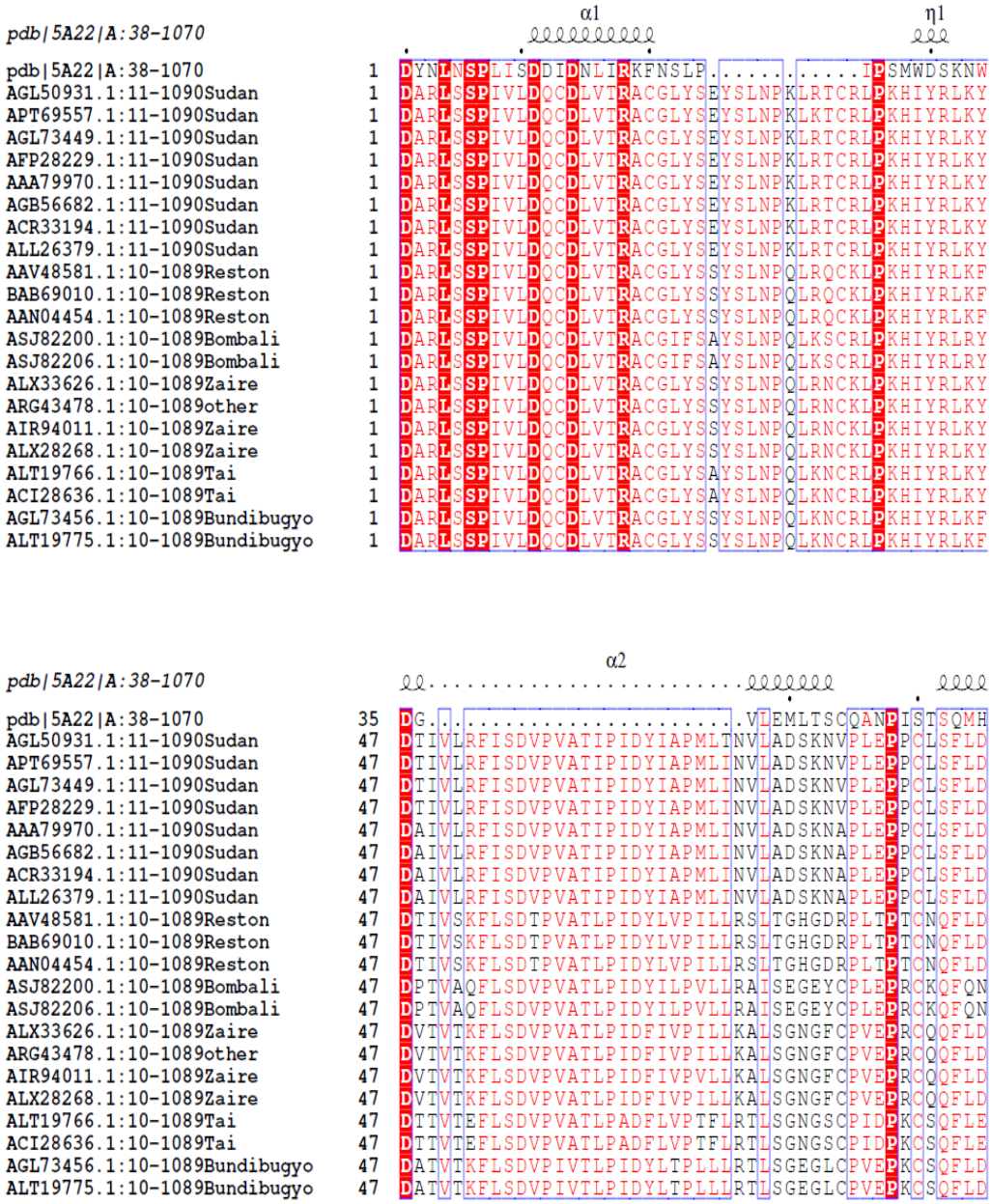

Fig.1. Sequence alignment for the 21 RdRp Ebola virus. The alignment is performed using CLUSTAL omega web server and visualized using ESPript software 3.0. The conserved amino acids are highlighted in red. The active site environment amino acids GDN are all conserved.

TABLE 1. Pairwise distance for EBOV polymerase sequence.

ACR33194 Sudan																								
AGB56682 Sudan	0.0019																							
ALL26379 Sudan	0.0009	0.0009																						
APT69557 Sudan	0.0074	0.0056	0.0065																					
AGL50931 Sudan	0.0074	0.0056	0.0065	0.0037																				
AGL73449 Sudan	0.0065	0.0046	0.0056	0.0028	0.0028																			
AFP28229 Sudan	0.0056	0.0037	0.0046	0.0019	0.0019	0.0009																		
AAA79970 Sudan	0.0056	0.0037	0.0046	0.0093	0.0093	0.0084	0.0074																	
ASJ82200 Bombali	0.2003	0.1980	0.1991	0.1946	0.1980	0.1969	0.1969	0.2014																
ASJ82206 Bombali	0.1980	0.1957	0.1969	0.1924	0.1957	0.1946	0.1946	0.1991	0.0037															
AGL73456 Bundibugyo	0.2094	0.2071	0.2082	0.2048	0.2082	0.2071	0.2071	0.2116	0.1779	0.1757														
ALT19775 Bundibugyo	0.2082	0.2059	0.2071	0.2037	0.2071	0.2059	0.2059	0.2105	0.1790	0.1768	0.0028													
ALX33826 Zaire	0.2003	0.1980	0.1991	0.1969	0.1969	0.1969	0.1969	0.2025	0.1801	0.1779	0.1367	0.1357												
AIR94011 Zaire	0.2014	0.1991	0.2003	0.1980	0.1980	0.1980	0.1980	0.2037	0.1757	0.1735	0.1367	0.1357	0.0093											
ALX28268 Zaire	0.1991	0.1991	0.1980	0.1980	0.1980	0.1980	0.1980	0.2037	0.1779	0.1757	0.1378	0.1367	0.0084	0.0028										
BAB69010 Reston	0.2014	0.1991	0.2003	0.2003	0.2014	0.2014	0.2003	0.2025	0.1980	0.1946	0.1901	0.1901	0.1890	0.1901	0.1901									
AAV48581 Reston	0.2025	0.2003	0.2014	0.2014	0.2025	0.2025	0.2014	0.2037	0.2025	0.1991	0.1913	0.1913	0.1901	0.1913	0.1913	0.0074								
AAN04454 Reston	0.2003	0.1980	0.1991	0.1991	0.2003	0.2003	0.1991	0.2014	0.2003	0.1969	0.1890	0.1890	0.1879	0.1890	0.1890	0.0046	0.0046							
ACI28636 Tai_Forest	0.2116	0.2094	0.2105	0.2048	0.2082	0.2071	0.2071	0.2139	0.1823	0.1801	0.0972	0.0961	0.1367	0.1388	0.1399	0.1969	0.1980	0.1957						
ALT19766 Tai_Forest	0.2105	0.2082	0.2094	0.2037	0.2071	0.2059	0.2059	0.2128	0.1812	0.1790	0.0961	0.0951	0.1357	0.1378	0.1388	0.1957	0.1969	0.1946	0.0009					
ARG43478 synthetic construct	0.1991	0.1969	0.1980	0.1957	0.1957	0.1957	0.1957	0.2014	0.1757	0.1735	0.1367	0.1357	0.0093	0.0056	0.0046	0.1890	0.1901	0.1879	0.1399	0.1388				

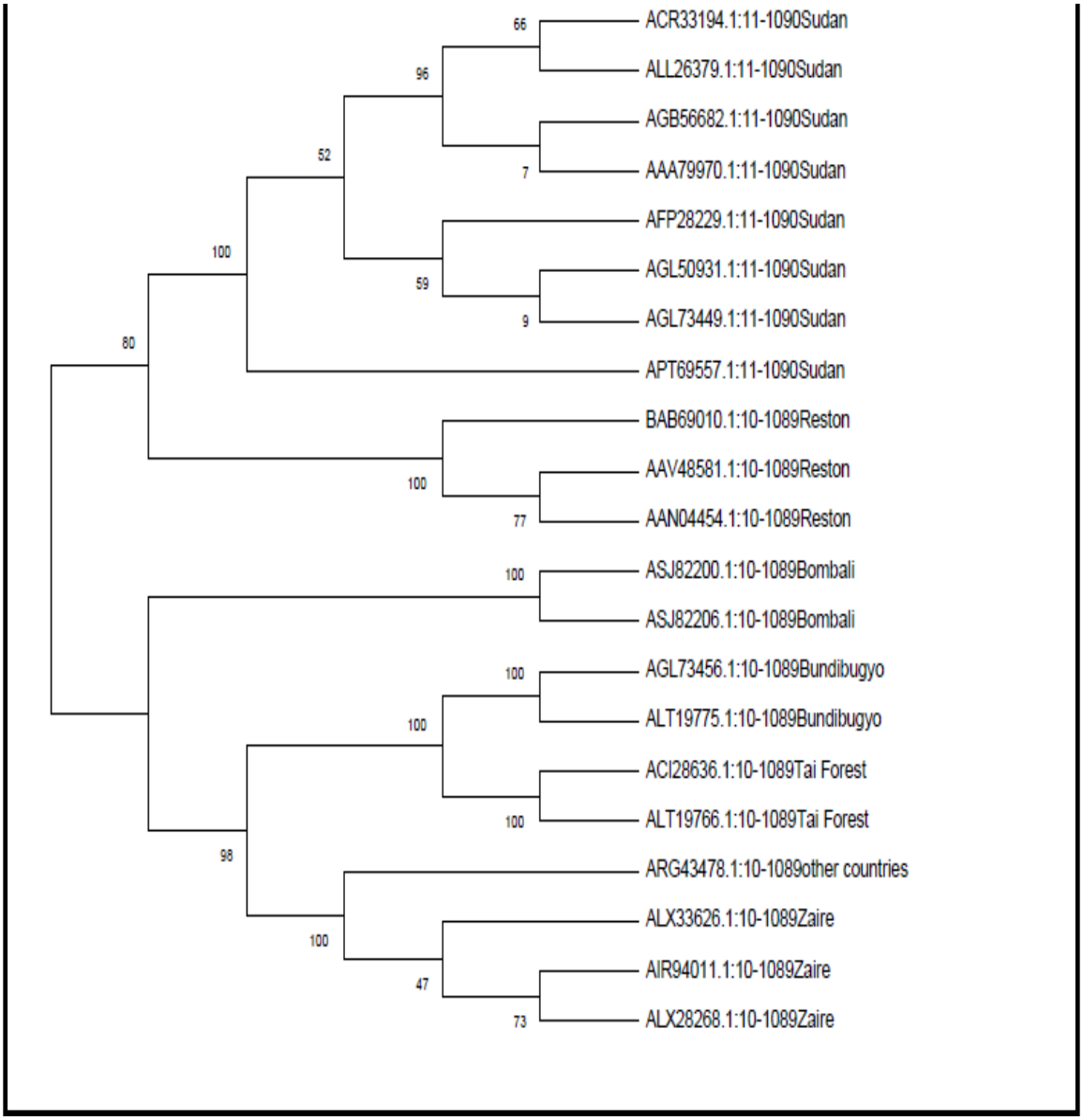

Fig. 2. A phylograph clarifying the evolutionary difference of RdRp Ebola virus. This phylogenetic tree is constructed using MEGA software. This graph is in the form of Cladograph.

Phylogenetic tree

Phylogenetic tree is inferred from the alignment as described in Materials and methods. A consensus tree inferred using the Maximum likelihood statistical method, bootstrap methods (test of phylogeny) with 100 replications, LG model and others with default parameters (Figure 2).

The aligned sequences of EBOV can be divided into two supergroup and branched into four lineages. The first lineage includes two subgroups; Sudan and Reston. The second group contains three subgroup; Bombali, Zaire and Tai Forest & Bundibugyo. Synthetic construct lies in the same subgroup of Zaire which indicates that there are fewer mutations than the synthetic construct and Zaire sequences. Tai Forest and Interestingly, the Sudan APT69557.1 sequence that released in January 2017 showed that different lineage compared to other Sudan sequences published in the period (August 2002 to January 2016). The same result is also reported for Zaire Sequence ALX33626.1 which released in November 2018. This indicates a new emerged mutated isoform of the EBOV RdRp.

Conclusion

EBOV RdRp active site is conserved among different sequence. The sequences analysis in the present study leads to RdRp inhibitors of other viruses such as HCV can be used to target EBOV L polymerase. Getting the 3D structure of EBOV L polymerase is the next step to test such polymerase inhibitors.

Among the observations of the phylogenetic analysis; sequences are occurred leading to distantly mutated isoforms and this should be as a target for future investigation.

Reference

1. Curtis N. Viral Haemorrhagic Fevers Caused by Lassa, Ebola and Marburg Viruses. 2006:35-44.
2. Kourtis AP, Appelgren K, Chevalier MS, McElroy A. Ebola Virus Disease. *Pediatr Infect Dis J*. 2015;34(8):893-897.
3. Falasca L, Agrati C, Petrosillo N, et al. Molecular mechanisms of Ebola virus pathogenesis: Focus on cell death. *Cell Death Differ*. 2015;22(8):1250-1259.
4. Teimoori S, Seesuay W, Jittavisutthikul S, et al. Human transbodies to VP40 inhibit cellular egress of Ebola virus-like particles. *Biochem Biophys Res Commun*. 2016;479(2):245-252.
5. Nelson E V., Pacheco JR, Hume AJ, et al. An RNA polymerase II-driven Ebola virus minigenome system as an advanced tool for antiviral drug screening. *Antiviral Res*. 2017;146:21-27.
6. Glanzer JG, Byrne BM, McCoy AM, James BJ, Frank JD, Oakley GG. In silico and in vitro methods to identify ebola virus VP35-dsRNA inhibitors. *Bioorganic Med Chem*. 2016;24(21):5388-5392.
7. Ilkhani H, Farhad S. A novel electrochemical DNA biosensor for Ebola virus detection. *Anal Biochem*. 2018;557(May):151-155.
8. Kadanali A. An overview of Ebola virus disease. *North Clin Istanbul*. 2016;2(1):81-86. doi:10.14744/nci.2015.97269
9. Tchesnokov EP, Raeisimakiani P, Ngure M, Marchant D, Götte M. Recombinant RNA-Dependent RNA Polymerase Complex of Ebola Virus. *Sci Rep*. 2018;8(1):1-9.
10. Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol*. 2011;7(539).
11. Higgins DG, Sharp PM. CL U S T AL : a package for performing multiple sequence alignment on a microcomputer. 1988;73:237-244.
12. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007;23(21):2947-2948.
13. Gouet P, Courcelle E, Stuart DI, Métoz F. ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics*. 1999;15(4):305-308.
14. Gouet P, Robert X, Courcelle E. ESPript/ ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins. *Nucleic Acids Res*. 2003;31(13):3320-3323.
15. Kabsch W, Sander C. Protein Secondary Structure: Pattern Recognition of Hydrogen-Bonded and Geometrical Features. 1983;22:2577-2637.
16. Frishman D, Argos P. Knowledge based protein secondary structure assignment. *Proteins Struct Funct Bioinforma*. 1995;23(4):566-579.
17. Elfiky AA, Elshemey WM, Gawad WA, Desoky OS. Molecular modeling comparison of the performance of NS5b polymerase inhibitor (PSI-7977) on prevalent HCV genotypes. *Protein J*. 2013;32(1):75-80.
18. Kumar S, Nei M, Dudley J, Tamura K. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform*. 2008;9(4):299-306.

(Recieved 28/9/2019;
accepted 13/10/2019)

دراسة التحليل التطورى والتسلسلى لأنزيم البلمره الرنا المعتمدة على الرنا لفيرس الإيبولا

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يعتبر فيروس إيبولا (EBOV) من الفيروسات التي إنتشرت على مدي العقود الأربعة الماضية وكان أكثر الأمراض فتكاً في عام 2015 في غرب إفريقيا ، مما أدي إلى وفاة أكثر من 10000 شخص. يصيب فيروس الايبولا كبد المريض عند حدوث تلامس مباشر مع سوائل الجسم الملوثة بالمرض أو دم المريض.

يعتبر انزيم البلمرة الفيروسى L (Polymerase L) أحد أهم البروتينات المسئولة عن عملية استنساخ الحمض الريبوزومى الفيروسى (RNA).

وقد نجحت مثبطات انزيم البلمرة الفيروسى (Polymerase Inhibitor) فى تثبيط استنساخ وعدوى الفيروسات كما حدث فى التهاب الكبد الوبائى المزمن فيروس C (HCV).

تحتوي قاعدة بيانات البروتين الخاصة بالمركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI) على 2123 تسلسلا لإنزيم البلمرة الفيروسى L (Polymerase L) .

في الدراسة الحاليه ، يتم استخدام التحليل التسلسلي (Sequence Analysis) والتحليل التطورى (phylogenetic analysis) لفهم التسلسلات غير المكررة التى تأتي من بلدان مختلفة.

استنادا إلى نموذج التشابه التسلسلى (Sequence Similarity) تم استخدام البنية ثلاثية الأبعاد لفيروس التهاب الفم الحويصلي المعروف التركيب الثلاثى الأبعاد له مسبقا (PDB ID: 5A22) في هذه الدراسة لمعرفة الموقع النشط(Active site) لأنزيم البلمره RNA المعتمد على RNA لفيروس الايبولا (EBOV RdRp).

أوضحت نتائج التحليل التطورى (phylogenetic analysis) ان هناك اثنان من التسلسلات من نفس المجموعة الجزيئية (Subgroup) يوجد بينهم طفرات و اختلاف فى توزيع الأحماض الأمينية مما يؤدى الى اقتراح احتمالية ظهور انواع جديدة من فيروس الإيبولا.

تم فى هذة الدراسة تحديد الموقع النشط لإنزيم البلمرة (Polymerase L) وهو (GDN) مما يتيح فرصة تجربة مثبطات انزيم البلمرة الفيروسى (Polymerase Inhibitor) المستخدمة على فيروسات أخرى سابقا على فيروس الإيبولا.