BIO F242

Introduction To Bioinformatics

END-SEMESTER REPORT

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Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000040705/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), and envelope protein (E) genes, complete cds; membran…

Task 1: Detail Introduction of gene and its functions

Gene Name: surface glycoprotein UIT12537.1

Locus: OM250521 29849 bp RNA linear VRL 13-JAN-2022

Definition: Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000040705/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), and envelope protein (E) genes, complete cds; membrane glycoprotein (M) gene, partial cds; and ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds.

Accession: OM250521

Version: OM250521.1

DBLINK BioProject: PRJNA750736

BioSample: SAMN24854787

Source: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Organism: Severe acute respiratory syndrome coronavirus 2

Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes;

Nidovirales; Cornidovirineae; Coronaviridae; Orthocoronavirinae;

Betacoronavirus; Sarbecovirus

This gene is a severe acute respiratory syndrome coronavirus 2 isolate and is a genomic RNA. Its isolation source is clinical. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA virus responsible for the Covid-19 pandemic. The positive-stranded RNA genome presents a 5’-cap and 3’-polyA tail, allowing its translation from host machinery. Similarly, to other CoVs, at the 5’-end, frameshift between Orfs, Orf1a and Orf1b leads to the production of two polypeptides that undergo proteolytic processing to produce 16 non-structural proteins, which direct various stages of the virus infection cycle such as virus assembly, transcription, replication, host control etc. The 3’-end encodes for structural proteins: S (spike glycoprotein), N (nucleocapsid protein), M (membrane protein) and E (Envelope

protein). The nucleocapsid protein binds to the viral genome and aids in the packing of the genome against the surface of the envelope. The other three structural proteins constitute the viral envelope. The 3'-end also encodes for the nine accessory proteins such as Orf3a, Orf3b, Orf6, Orf7a, Orf7b, Orf8, Orf9b, Orf9c, and Orf10. Accessory proteins play a key role in the virulence and host interaction of the SARS-CoV-2 virus.

The Spike Glycoprotein is a glycosylated type-I membrane protein existing in the trimeric pre-fusion form which is later cleaved into two sub-units S1 and S2, by a host Furin protease. The N-terminal S1 subunit consists of a Receptor-binding domain which helps in binding to the host cell receptor called angiotensin-converting enzyme 2 (ACE2). The binding of RBD to ACE2 and cleavage of the S2 subunit by host serine protease TMPRSS2 leads to dissociation between S1 and S2 subunits. The dissociation of subunits conformationally changes S2. The conformationally changed form of S2 is needed for the fusion of host and viral membranes and host entry. S protein works in tandem with other structural proteins N, E and M during membrane fusion in coronaviruses. The lower variability of the S2 sub fusion subunit makes it an ideal target for the development of inhibitors targeting viral entry.

The Envelope protein plays a vital role in viral morphogenesis and assembly. E protein acts as viroporins which assemble themselves into protein-lipid pores of the host responsible for forming membrane and are involved in ion transport. Interaction between the C-terminus of E and M proteins recruits E protein to the intermediary compartment of the Golgi-Endoplasmic Reticulum. This positioning of E-protein in the Golgi-ER intermediary compartment initiates the budding of the virus into the host cells. M proteins are the most abundant proteins in CoVs and attribute distinct shapes to the virus. M proteins also act as scaffolding platform to recruit other structural proteins and promotes membrane curvature during the budding of the virion. Nucleocapsid (N) protein is responsible for packaging viral RNA into ribonucleocapsid. N protein mediates viral assembly by interacting with the viral genome and M protein and boosting the replication and transcription of viral RNA. The N protein of SARS CoV-2 inhibits the activity of the cyclin-CDK complex, which hypo phosphorylates retinoblastoma protein and hence, inhibits the S phase progression of the cell cycle.

The accessory protein Orf3a forms an ion channel causing calcium influx. The ion channel is observed to be selective towards K^+ and Ca^{2+} over Na^+ in vitro. Orf3a channel-forming activity is associated with its pro-apoptotic activity in the SARS-CoV-2 virus. ORF3a also interacts with TRAF3, which activates ASC ubiquitination leading to caspase 1 activation and IL-1β maturation. ORF3a additionally interacts with structural proteins N, M and S to aid virus budding. ORF1ab contains overlapping open reading frames to encode polyproteins PP1ab and PP1a. ORF1a is the first open reading frame at the 5'-end of the genome. ORF1a, together with ORF2ab, occupies about 2/3 of the SARS-Cov-2 genome and is translated from 5'-capped RNA by cap-dependent translation. PP1ab is produced when a programmed ribosomal frameshift allows reading the stop codon, which terminates ORF-1a. The frameshift takes place in a slippery sequence followed by pseudoknot RNA secondary structure. These polyproteins are cleaved to yield 13 to 17 nonstructural proteins or NSPs. They release nonstructural proteins by auto-proteolysis and due to the action of internal cysteine protease domains. PP1a protein

contains nonstructural proteins nsp1 to nsp11, whereas pp1ab protein contains nsp1-10 and nsp12-16. Proteolytic processing is performed by two proteases: papin like protease domain located in multidomain protein nsp3 cleaving to nsp4. The second protease, 3CL protease, performs the remaining nsp5 cleavages through the C-terminus of the polyprotein. The C-terminal components of pp1ab polyprotein, that is, nsp12-16, have core enzymes for viral replication. After proteolytic processing is completed, non-structural proteins form a large protein complex called replicase-transcriptase complex (RTC), responsible for gene replication and transcription.

Task 2: Download Gene sequence, mRNA sequence, protein sequence and protein structure (if available)

Gene Sequence retrieved from FASTA (DNA):

>OM250521.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000040705/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), and envelope protein (E) genes, complete cds; membrane glycoprotein (M) gene, partial cds; and ORF6 protein (ORF6), ORF7a protein (ORF7a), ORF7b (ORF7b), ORF8 protein (ORF8), nucleocapsid phosphoprotein (N), and ORF10 protein (ORF10) genes, complete cds

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CGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTA

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3. mRNA Sequence

mRNA Sequence (Obtained from Python sequence)

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GCGAUCAAAACAACGUCGGCCCCAAGGUUUACCCAAUAAUACUGCGUCUUGGUUCACCGC

UCUCACUCAACAUGGCAAGGAAGACCUUAAAUUCCCUCGAGGACAAGGCGUUCCAAUUAA
CACCAAUAGCAGUCCAGAUGACCAAAUUGGCUACUACCGAAGAGCUACCAGACGAAUUCG
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GGGAGCAGAGGCGGCAGUCAAGCCUCUUCUCGUUCCUCAUCACGUAGUCGCAACAGUUCA
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AUGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Protein Sequence (Using NCBI)

```
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 61 nvtwfhaihv sgtnvikrfd npvlpfndgv yfasteksni irgwifgttl dsktqslliv
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241 llalhxxxxx xxnsssgwta gaaayyvgyl qprtfllkyn engtitdavd caldplsetk
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1201 qelgkyeqyi kwpwyiwlgf iagliaivmv timlccmtsc csclkgccsc gscckfdedd
1261 sepvlkgvkl hyt

Longest Amino Acid Length as obtained in FGENESV

1. Paste the Sequence of Longest Amino Acid:

MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKDGTCGLVEVEKGV

LPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQYGRSGETLGVLVPHVGEIPVAYRK

VLLRKNGNKGAGGHSYGADLKSFDLGDELGTDPYEDFQENWNTKHSSGVTRELMRELNGG

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IIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSN

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QYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI

GKIQDSLSSTASALGKLQDVVNQXXXALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEV

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QCSKESNYITHKRTYGFVYENLHNWNCNFEASVTSSIVITSGDGTTSPISEHDYQIGGYT

EKWESGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEHVTFFIYNKIVDEPEEHVQIHTID

GSSGVVNPVMEPIYDEPTTTTSVPLLFARTRSMWSFNPETNILLNVPLHGTILTRPLLES

ELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYKLGASQRVAGIITTVAA

FHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIELCVDEAGSKSPIQYID

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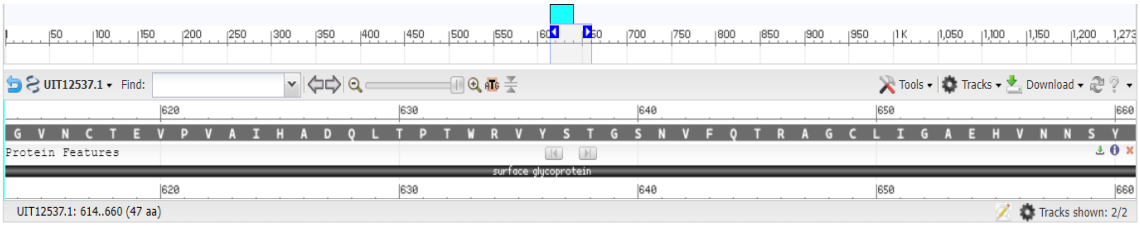
RLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGN

FGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDKDPN

FKDQVILLNKHIDAYKTFPPXEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSK

QLQQSMSSADSTQA

Since protein structure is not available, graphical summary of the protein sequence is as follows:



Conclusion and Inference:

1. **Genes and their Location:** This nucleotide sequence contains 11 different genes which are located at the following locations and following CDS

Gene Location	CDS	CDS Length
212..21501	join(212..13414,13414..21501)	join(212..13414,13414..21501) = 21289 bp
21509..25330	21509..25330	21509..25330 = 3821 bp
25339..26166	25339..26166	25339..26166 = 827 bp
26191..26418	26191..26418	26191..26418 = 227 bp

26469..>27123	26469..>27123	26469..>27123 = 654 bp
27148..27333	27148..27333	27148..27333 = 185 bp
27340..27705	27340..27705	27340..27705 = 365 bp
27702..27833	27702..27833	27702..27833 = 131 bp
27840..28205	27840..28205	27840..28205 = 365 bp
28220..29479	28220..29479	28220..29479 = 1259 bp
29504..29620	29504..29620	29504..29620 = 116 bp

2. **Exon Count and its Location:** Since viral genome, so no exons are present
3. **A, T, G. C Composition:** The given gene sequence of surface glycoprotein UIT12537.1 gene consists of:
    i. **A:** 8899
    ii. **T:** 9548
    iii. **G:** 5833
    iv. **C:** 5440
4. **Number of Start Codons (ATG):** 723
5. **Number of Stop Codons (TAG+ TGA + TAA)**: 1766
6. **Percentage GC Content**- 37.766759355422295%
7. **The Length of mRNA Transcript is** …29849…….. bp
8. **Total number of EcoRI are:** 9
9. **Total number of BamHI are:** 1
10. **Total HindIII are**: 17
11. **Intron-Exon Boundary at positions:** 212, 21501, 21509, 25330, 25339, 2616626191, 26418, 26469, 27123, 27148, 27333, 27340, 27705, 27702, 27833, 27840, 28205, 28220, 29479, 29504, 29620

Using FGENESV and GENSCAN

12. **Number of Isochore:** 1(0 - 43 C+G%)
13. **Number of genes:** 1 gene (12 coding sequences) in GENSCAN, 11 genes in FGENESV0, 12 genes in FGENESV
14. **Number of Initial exon** (ATG to 5' splice site): 1
15. **Number of Internal exon** (3' splice site to 5' splice site): 10
16. **Number of Terminal exon** (3' splice site to stop codon): 1
17. **Number of Single-exon gene (ATG to stop)**: 0
18. **Length of longest Promoter (TATA box / initation site):** 0 (no promoter region mentioned
19. **Length of longest poly-A signal** (consensus: AATAAA): 6
20. **Maximum Coding region score (tenth bit units):** 4112
21. **(No exon found) Maximum P: probability of exon (sum over all parses containing exon):** 0.975
22. **Length of Longest Amino Acid**: 7814 aa

By ORF Finder:

23. **The ORF that has Maximum Length:** ORF4
24. **The length of ORF that has Maximum Length:** 13218 nucleotides

25. **The location of START Codon in Maximum Length of ORF:** 212
26. **The location of STOP Codon in Maximum Length of ORF:** 13429
27. **The Direction of STRAND in Maximum Length of ORF:** Positive
28. **The Length of Amino Acid encoded by BIGGEST ORF:** 4405

Task 3: Self dot plot of gene, mRNA, protein sequence, and draw inference from your dot plots.

Self Dot-Plot for gene

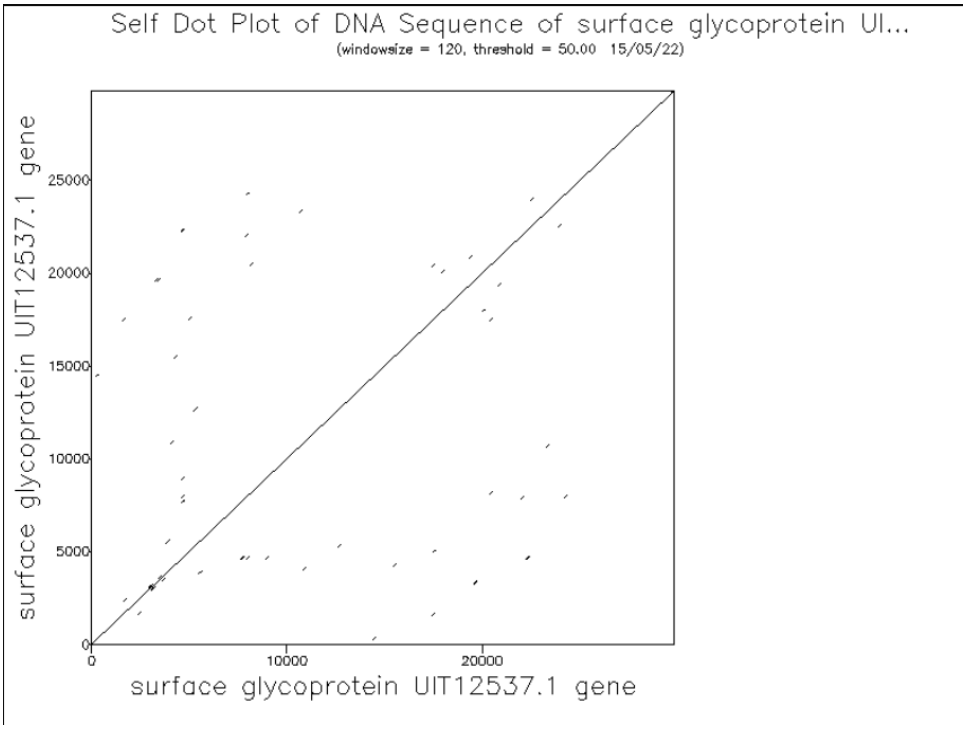


Figure 1: Self dot-match of Surface Glycoprotein UIT12537.1 gene

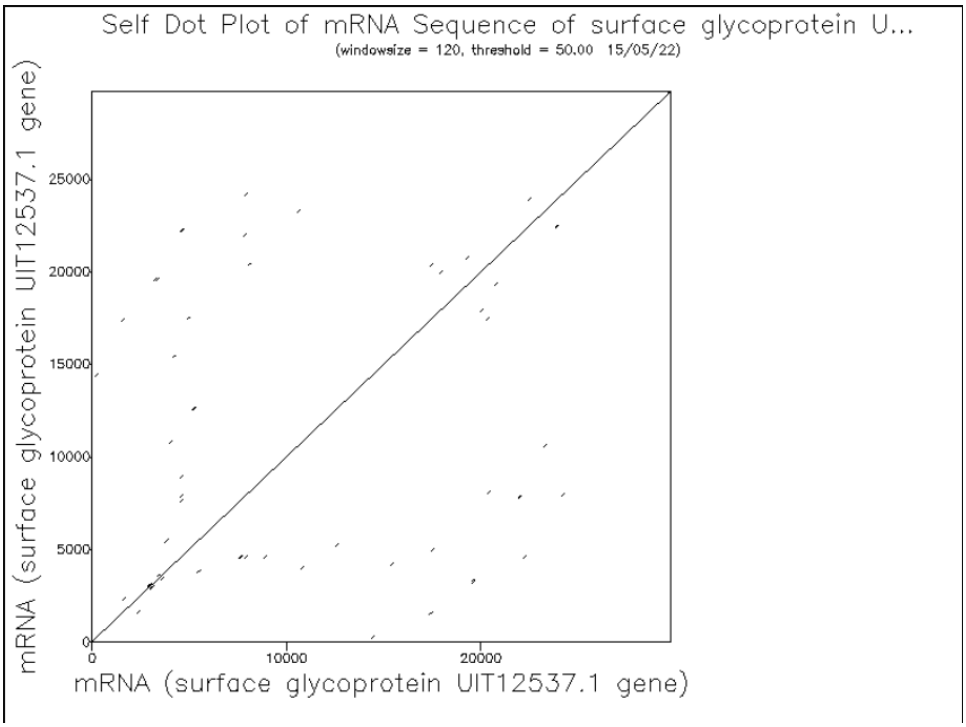


Figure 2: Self dot-match of Surface Glycoprotein UIT12537.1 mRNA Sequence

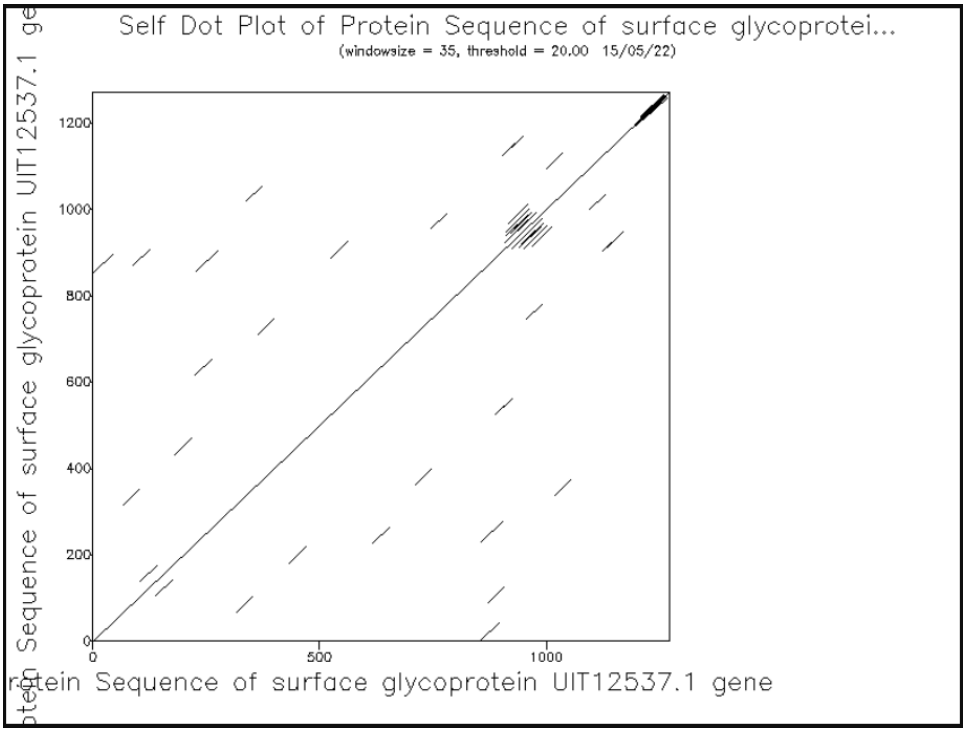


Figure 2: Self dot-match of Surface Glycoprotein UIT12537.1 Protein Sequence

Conclusion and Inference:

The main diagonal represents the sequence's alignment with itself. Since, here we have plotted self dot-plot of DNA, mRNA and protein, we observed a diagonal here.

1. The Pairwise Sequence Alignment (PSA) of Surface Glycoprotein UIT12537.1 gene with itself shows maximum alignment by selecting window size 120 and threshold 50.00.
2. The Pairwise Sequence Alignment (PSA) of Surface Glycoprotein UIT12537.1 gene with itself shows maximum alignment by selecting window size 120 and threshold 50.00.
3. The Pairwise Sequence Alignment (PSA) of Surface Glycoprotein UIT12537.1 gene with itself shows maximum alignment by selecting window size 35 and threshold 20.00. The repeats being parallel to the main diagonal means that the repeats are occurring in different places throughout the protein sequence but are not inverted.

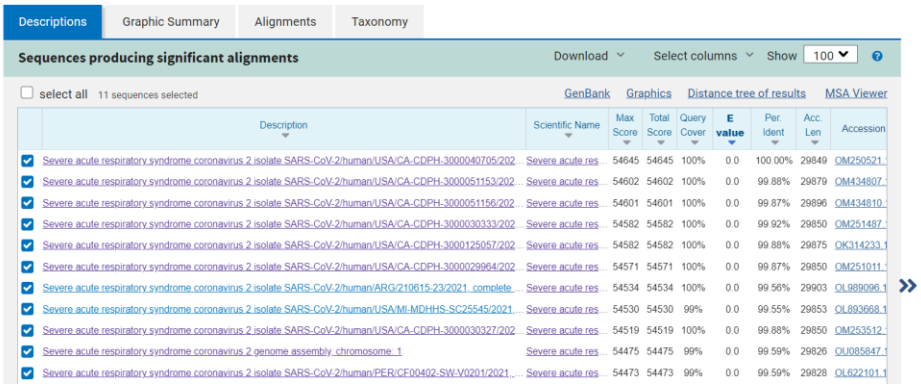
Task 4: BLAST your gene and protein sequence and find the top 5 eukaryotic and 5 prokaryotic genes and protein (from different species) sequences. Tabulate results mentioning score, E-value, % of identity, query coverage, etc.

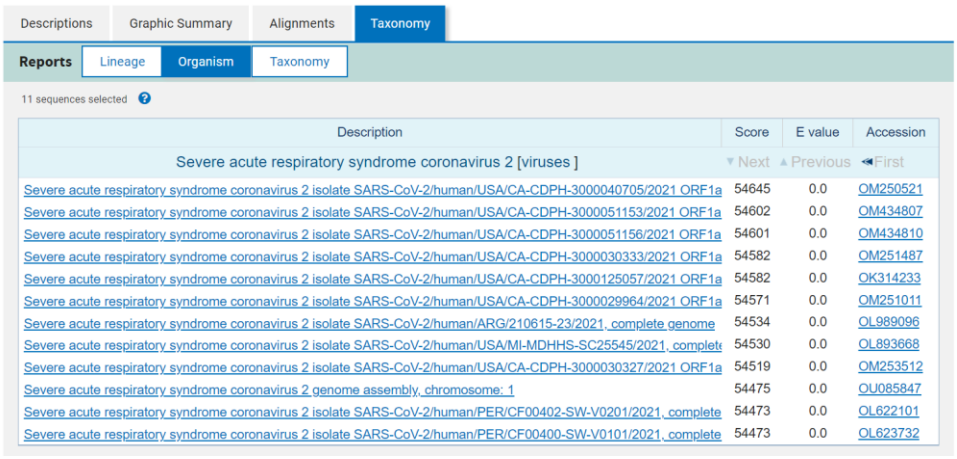
Since the gene belongs to SARS-CoV-2 belong to a virus, it does not share similarity with any prokaryote or eukaryote gene. Therefore, instead of any prokaryote or eukaryote, I have taken the top ten sequences most similar to the surface glycoprotein UIT12537.1 that appeared in BLAST (Megablast for similar sequences).

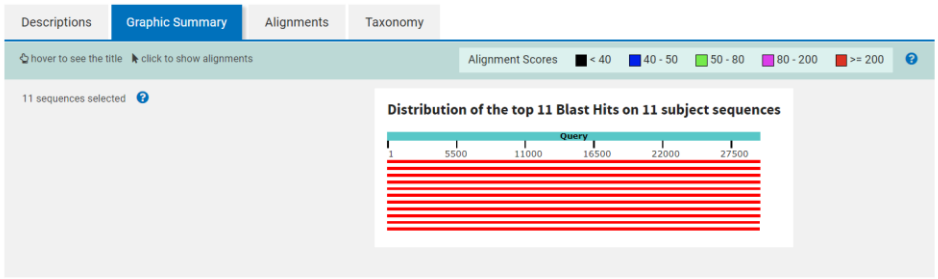
BLASTN

S.No	Accession Number	Max Score	Total Score	E-Value	Query Coverage	% Identity
1	OM434807.1	54602	54602	0.0	100%	99.88%
2	OM434810.1	54601	54601	0.0	100%	99.87%
3	OM251487.1	54582	54582	0.0	100%	99.92%
4	OK314233.1	54582	54582	0.0	100%	99.88%
5	OM251011.1	54571	54571	0.0	100%	99.87%
6	OL989096.1	54534	54534	0.0	100%	99.56%
7	OL893668.1	54530	54530	0.0	99%	99.55%
8	OM253512.1	54519	54519	0.0	100%	99.88%
9	OU085847.1	54475	54475	0.0	99%	99.59%
10	OL622101.1	54473	54473	0.0	99%	99.59%

Screenshots of BLASTN Interface and Results





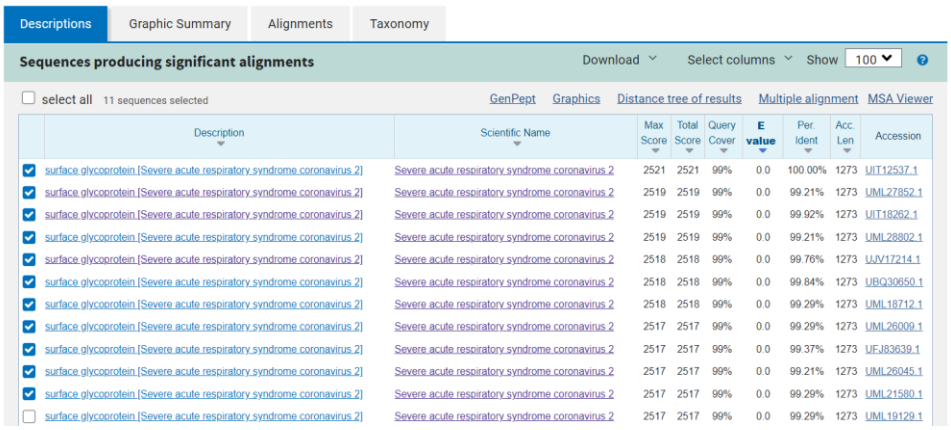


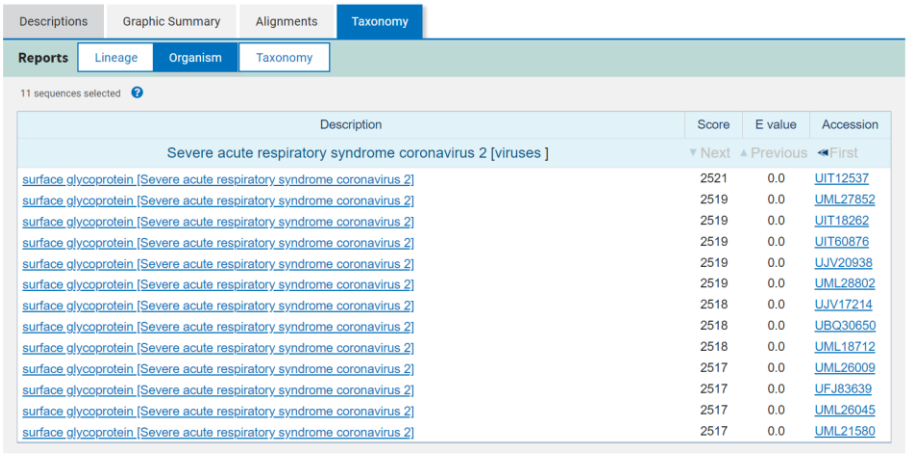
BLASTP

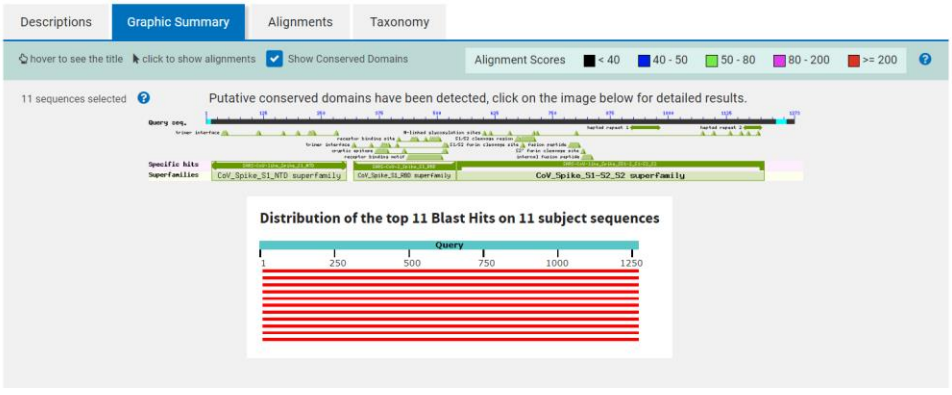
S.No	Accession Number	Max Score	Total Score	E-Value	Query Coverage	% Identity
1	UML27852.1	2519	2519	0.0	99.0%	99.21%
2	UIT18262.1	2519	2519	0.0	99.0%	99.92%
3	UML28802.1	2519	2519	0.0	99.0%	99.21%
4	UJV17214.1	2518	2518	0.0	99.0%	99.76%
5	UBQ30650.1	2518	2518	0.0	99.0%	99.84%
6	UML18712.1	2518	2518	0.0	99.0%	99.29%
7	UML26009.1	2517	2517	0.0	99.0%	99.29%
8	UFJ83639.1	2517	2517	0.0	99.0%	99.37%

9	UML26045.1	2517	2517	0.0	99.0%	99.21%
10	UML21580.1	2517	2517	0.0	99.0%	99.29%

Screenshots of BLASTP and Interface







Conclusion and Inference

As the highest score indicates highest similarity in BLAST, The Pairwise Sequence Alignment (PSA) using BLASTn of surface glycoprotein UIT12537.1 gene nucleotide sequence shows maximum alignment with **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000051153/2021 (OM434807.1) having Score 54562, E-value 0 and percentage similarity of 99.88%,** respectively.

The top ten organisms that are showing maximum similarity with surface glycoprotein UIT12537.1 gene

1. **SARS-CoV-2/human/USA/CA-CDPH-3000051153/2021 (OM434807.1)** having **E-value 0 and percentage similarity of 99.88%**, respectively.
2. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000051156/2021 (OM434810.1)** having E-value...**0.0**....and Percentage similarity...**99.87%**..... respectively.
3. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000030333/2021 (OM251487.1)** having E-value...**0.0**....and Percentage similarity...**99.92%**..... respectively.
4. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000125057/2021 (OK314233.1)** having E-value...**0.0**....and Percentage similarity...**99.88%**..... respectively.
5. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000029964/2021 (OM251011.1)** having E-value...**0.0**....and Percentage similarity...**99.87%**..... respectively.
6. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/ARG/210615-23/2021, complete genome (OL989096.1)** having E-value...**0.0**....and Percentage similarity...**99.56%**..... respectively.
7. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/MI-MDHHS-SC25545/2021, complete genome (OL893668.1)** having E-value...**0.0**....and Percentage similarity...**99.55%**..... respectively.
8. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/CA-CDPH-3000040705/2021 (OM253512)** having E-value...**0.0**....and Percentage similarity...**99.88%**..... respectively.
9. **Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1 (OU085847.1)** having E-value...**0.0**....and Percentage similarity...**99.59%**..... respectively.
10. **Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/PER/CF00402-SW-V0201/2021 (OL622101.1)** having E-value...**0.0**....and Percentage similarity...**99.59%**..... respectively.

B) The Pairwise Sequence Alignment (PSA) using BLASTp of surface glycoprotein UIT12537.1 gene amino acid sequence shows maximum alignment with **Surface Glycoprotein UML27852.1,**

Surface Glycoprotein UIT18262.1 and Surface Glycoprotein UML28802.1 with score 2519 E-value 0 and percentage similarity of 79.37%, respectively.

The top protein sequences that are showing maximum similarity with Surface Glycoprotein UIT12537.1

11. **Surface Glycoprotein UML27852.1** having E-value...**0.0....** and Percentage similarity.....99.21**%**.... respectively
12. **Surface Glycoprotein UIT18262.1** having E-value...**0.0..**.... and Percentage similarity.....**99.92%**....respectively
13. **Surface Glycorotein UML28802.1** having E-value...**0.0...**. and Percentage similarity...**..99.21%**.... respectively.
14. **Surface Glycoprotein UJV17214.1** having E-value...**0.0..**. and Percentage similarity**....99.76%.**.....respectively
15. **Surface Glycoprotein UBQ30650.1** having E-value**....0.0.**.... and percentage similarity.....**99.84%**...respectively
16. **Surface Glycoprotein UBQ30650.1** having E-value**....0.0.**.... and percentage similarity.....**99.29%**...respectively
17. **Surface Glycoprotein UML18712.1** having E-value**....0.0.**.... and percentage similarity.....**99.29%**...respectively
18. **Surface Glycoprotein UML26009.1** having E-value**....0.0.**.... and percentage similarity.....**99.37%**...respectively
19. **Surface Glycoprotein UFJ83639.1** having E-value**....0.0.**.... and percentage similarity.....**99.21%**...respectively
20. **Surface Glycoprotein UML21580.1** having E-value**....0.0.**.... and percentage similarity.....**99.29%**...respectively

Task 5: Perform pairwise global alignment (Needleman-Wunch, EMBOSS) of your query sequence (both DNA and protein) with all 10 sequences which you retrieved by BLAST search. Tabulate your result by mentioning score and % of identity etc.

Pairwise global alignment (Needleman-Wunch using NCBI and EMBOSS) of DNA sequence tabulation

SR. No	Accession No.	Needleman-Wunch Score	EMBOSS Needle Score	Needleman-Wunch Percent Identity	EMBOSS Needle Percent Identity
1.	OM434807.1	58943	60423.0	100.00%	99.8%

2.	OM434810.1	58909	60598.5	100.00%	99.7%
3.	OM251487.1	58981	60530.5	100.00%	99.9%
4.	OK314233.1	58930	60587.0	100.00%	99.8%
5.	OM251011.1	58971	60786.5	100.00%	99.9%
6.	OL989096.1	58825	59992.5	99.00%	99.4%
7.	OL893668.1	58887	61011.5	100.00%	99.5%
8.	OM253512.1	58896	60255.5	100.00%	99.9%
9.	OU085847.1	58720	60568.0	99.00%	99.3%
10.	OL622101.1	58710	59543.5	99.00%	99.3%

Pairwise global alignment (Needleman-Wunch using NCBI and EMBOSS) of Protein sequence tabulation

SR.No	**Accession No.**	**Needleman-Wunch Score**	**EMBOSS Needle Score**	**Needleman-Wunch Percent Identity**	**EMBOSS Needle Percent Identity**
1.	UML27852.1	6629	6631.0	99.0%	99.2%
2.	UIT18262.1	6629	6629.0	100.0%	99.9%
3.	UML28802.1	6629	6632.0	99.00%	99.2%
4.	UJV17214.1	6629	6629.0	100.00%	99.8%
5.	UBQ30650.1	6629	6629.0	100.00%	99.8%
6.	UML18712.1	6629	6631.0	99.00%	99.3%
7.	UML26009.1	6621	6622.0	99.00%	99.3%
8.	UFJ83639.1	6624	6625.0	99.00%	99.4%
9.	UML26045.1	6621	6622.0	99.00%	99.2%

10.	UML21580.1	6624	6625.0	99.00%	99.3%

Conclusion/Inference

As the per cent identity gives the percentage of identical matches between the two sequences over the reported aligned region (including any gaps in the length), therefore following conclusions are derived.

1. For Nucleotide: The Pairwise Sequence Alignment (PSA) using **EMBOSS NEEDLE** between set of the DNA sequences as retrieved by BlastN shows maximum similarity of **Surface Glycoprotein UIT12537.**1 (OM250521) with **SARS-CoV-2 Isolate (Accession Number: OM253512.1) showing 99.9%** identity as shown in the table.

   If PSA is performed using **NCBI Needle-Wunch Method**, the maximum similarity of **Surface Glycoprotein UIT12537.1 (OM250521.1) is with genes with accession number: OM434807.1, OM434810.1, OM251487.1, OK314233.1, OM251011.1, OL893688.1, OM253512.1 each showing 100% percentage identity** as shown in the table.

2. For Protein: The Pairwise Sequence Alignment (PSA) using **EMBOSS NEEDLE** between set of protein sequences as retrieved by BlastP shows maximum similarity of **Surface Glycoprotein UIT12537.**1 with **surface glycoprotein UIT18262.1** with **99.9%** identity as shown in the table.

   If PSA is performed using **NCBI Needle-Wunch Method**, the maximum similarity of **Surface Glycoprotein UIT12537.1 is with Surface Glycoprotein UIT18262.1, Surface Glycoprotein UJV17214.1 and UBQ30650.1 with 100% identity.**

Task 6: Make multiple sequence alignment (CLUSTALW, or any other popular algorithm or software) of your query with the other 20 sequences that you retrieved through blast (both DNA and protein). Analyze your result by mentioning consensus sequences and motifs that you may identify through MSA

Results of Multiple Sequence Alignment of DNA Sequences using CLUSTALW

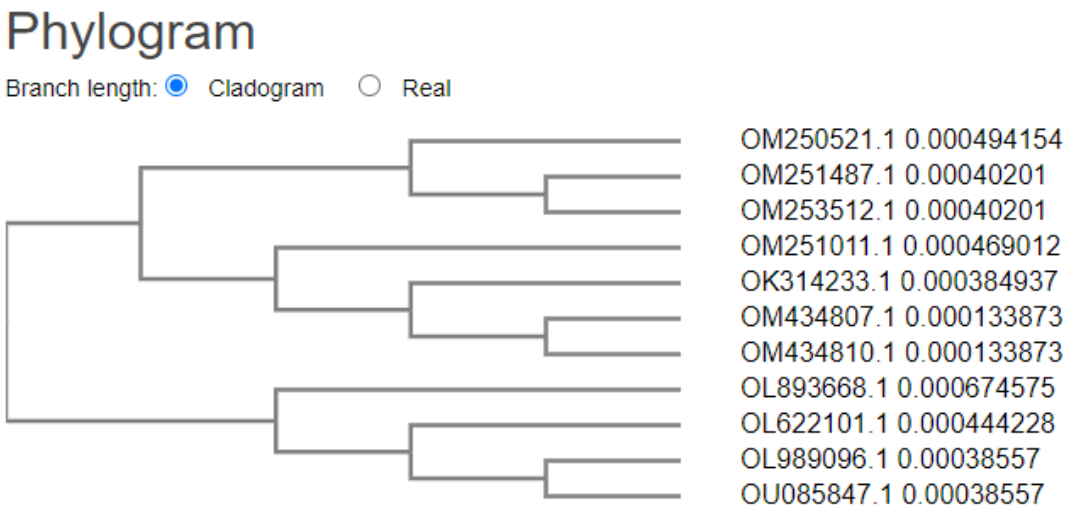


Fig: Phylogram or Guide Tree of Nucleotide Sequence obtained in ClustalW

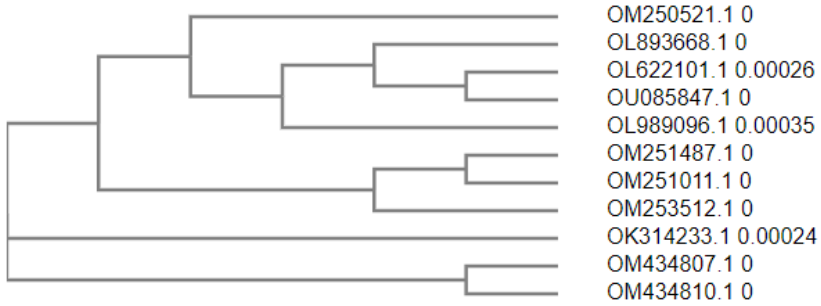


Fig: Phylogenetic Tree of Nucleotide Sequence obtained in ClustalW

Consensus Sequence generated by EMBOSS Cons for given set of nucleotides

>EMBOSS0001

AnATCnnnnnTCTAnnnnnnCTTTnAAnTnnnTnTGnCTnnnAnnCGnnTnnAnnCTTnG
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Results of Multiple Sequence Alignment of Protein Sequences using CLUSTALW

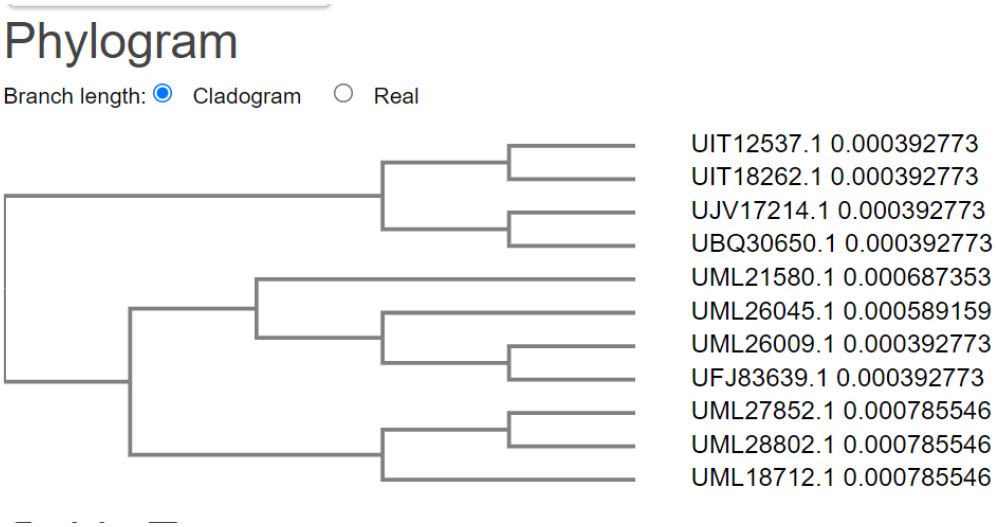

Fig: Phylogram or Guide Tree of Protein Sequence obtained in ClustalW

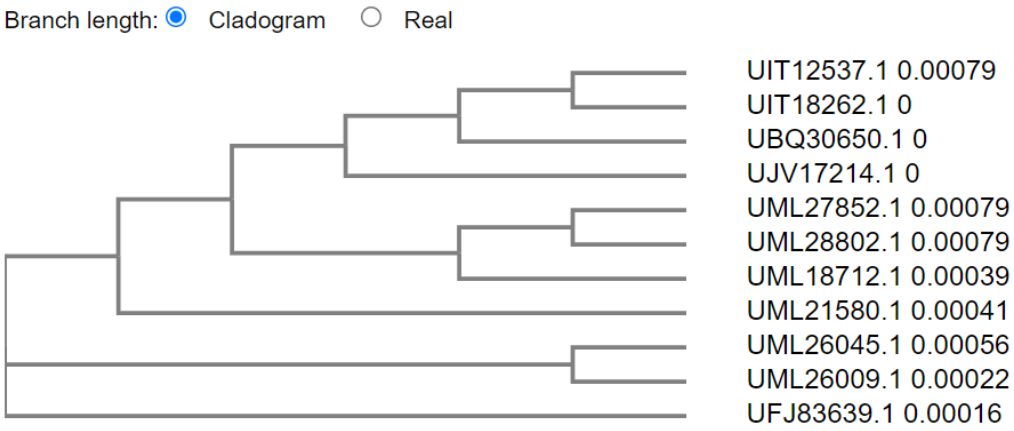

Fig: Phylogenetic Tree of Protein Sequence obtained in ClustalW

Consensus Sequence of given set of proteins as generated in EMBOSS Cons

>EMBOSS0001

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ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI

SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE

VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC

LGDIAARDLICAQKFNGLNVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM

QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALN

TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA

SANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPA

ICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDP

LQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL

QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD

SEPVLKGVKLHYT

Conclusion/Inference

A) For DNA Sequence: The Multiple Sequence Alignment (MSA) using Clustal Omega of surface glycoprotein UIT12537.1 gene sequence with sequences obtained in the Blastn shows Presence of **Conserved regions (*)**. The guided tree in the form of cladogram (using distance matrices) showed that are closely related with a minimum distance of **0.000133873**.

The Real tree shows that the number of mutational steps is highest in genes **with accession number OM434807.1 and OM434810.1** both have values as **.000133873.**

The Phylogenetic tree (neighbour-joining method) shows that **genes with accession numbers OL622101.1 and OU085847.1** are recent descendants with values of **0.00026 and 0**, respectively while genes with **accession number OK314233.1 and ancestor of genes (OM434807.1 and OM434810.1) have diverged** during early phage of evolution having **0.00024 and 0 value** respectively.

B) For Protein Sequence: The Multiple Sequence Alignment (MSA) using Clustal Omega of surface glycoprotein UIT12537.1 gene sequence with sequences obtained in the Blastp shows Presence of **Conserved regions (*)**. The guided tree in the form of cladogram (using distance matrices) showed that (**surface glycoprotein UIT12537.1 and surface glycoprotein UIT18262.1),** (**surface glycoprotein UJV17214.1 and surface glycoprotein UBQ30650.1),** (**surface glycoprotein UML26009.1 and surface glycoprotein UFJ83639.1),** are closely related with a minimum distance of **0.000392773**.

The Real tree shows that the number of mutational steps are highest in **surface glycoprotein UML26009.1** and **surface glycoprotein UFJ83639.1** having **0.000392773 and 0.000392773 value respectively.**

The Phylogenetic tree (Neighbor Joining method) shows that **surface glycoprotein UIT12537.1 and UIT18262.1** are recent descendants with values 0.00079 and 0 respectively while **surface glycoprotein UFJ83639.1** has been diverged during early phage of evolution having **0.00016** value.

Task 7: Draw MP, NJ, ML trees (with 500 bootstrap) with your multiple sequence alignment and compare your trees and draw inference regarding the evolution of your gene/protein.

MP, NJ, ML Trees (with 500 bootstrap) for nucleotide multiple sequence alignment

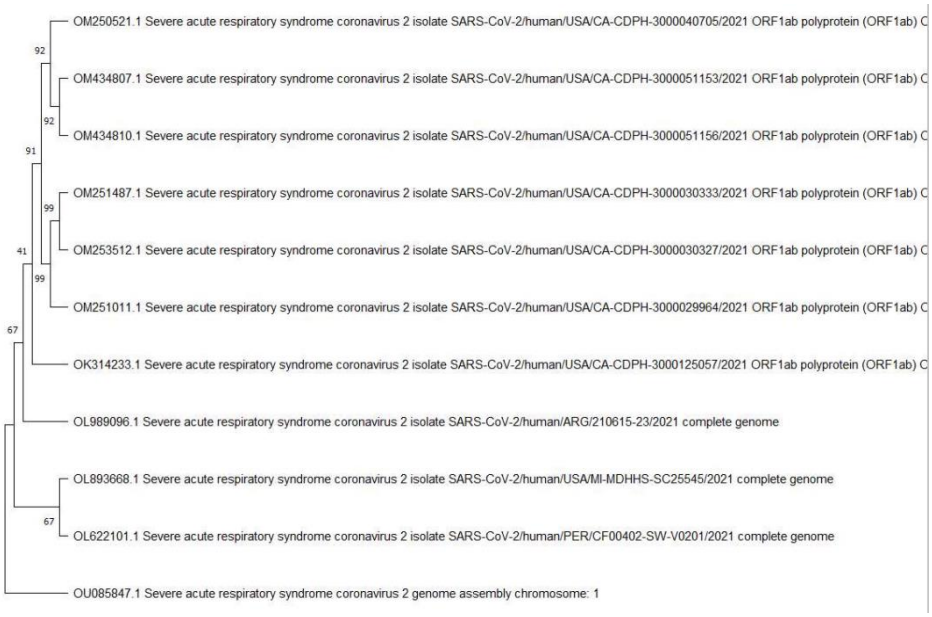

Fig: Phylogenetic Tree of given nucleotide sequence using Maximum Parsimony (MP)

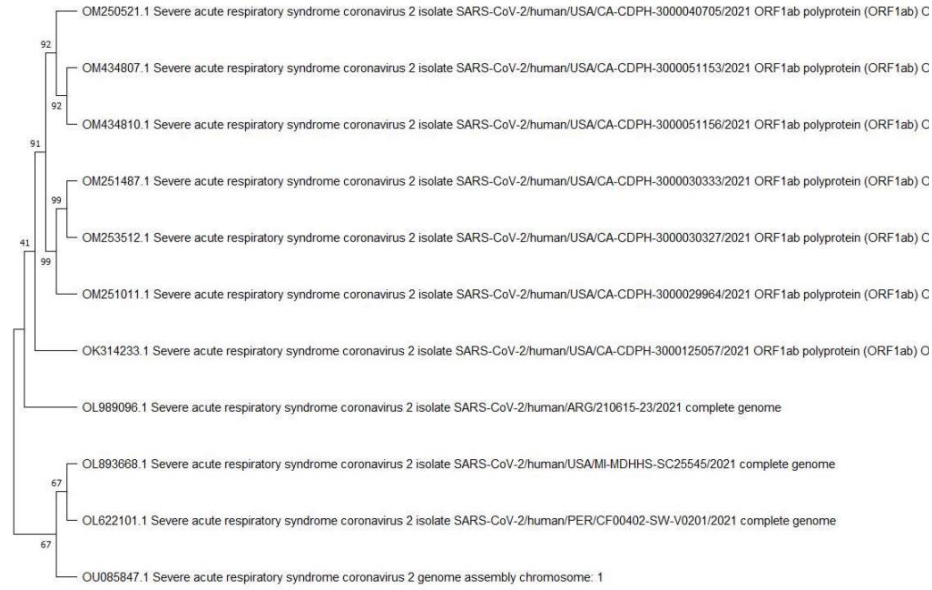

Fig: Phylogenetic Tree of given nucleotide sequence using Maximum Parsimony (MP) with bootstrap

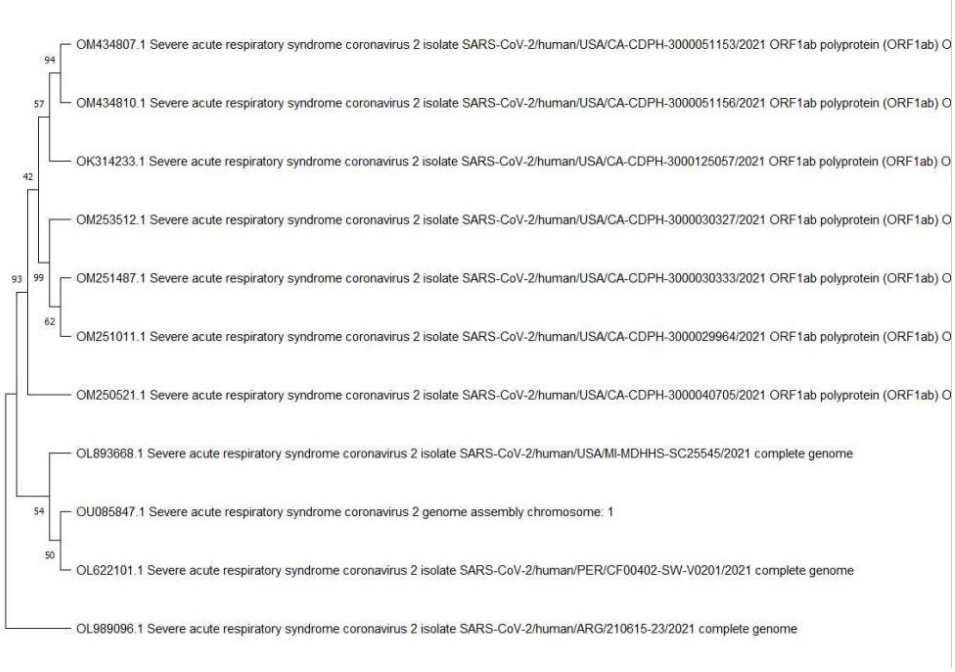

Fig: Phylogenetic Tree of given nucleotide sequence using Neighborhood-Joining Method

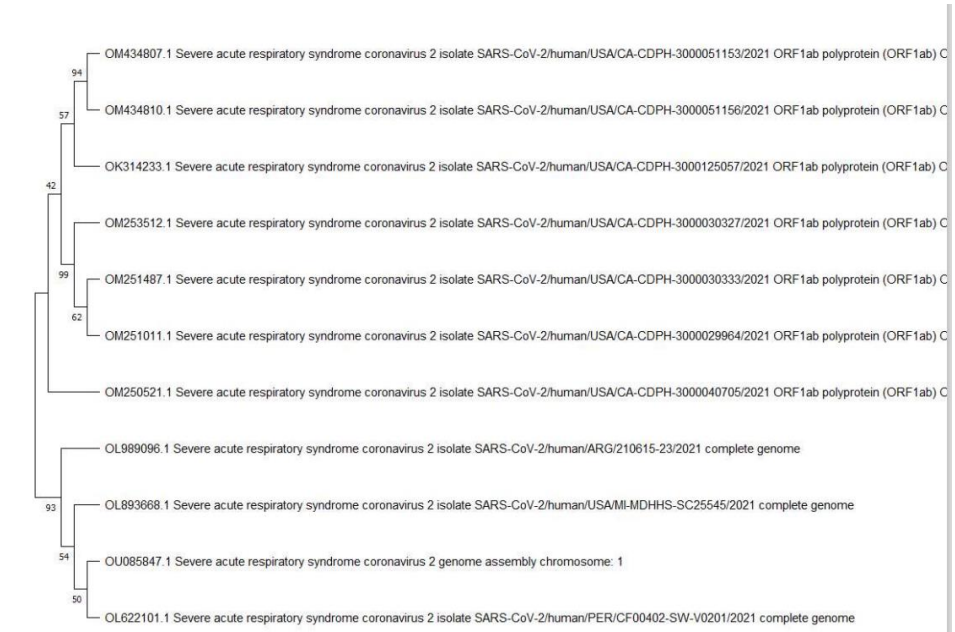

Fig: Phylogenetic Tree of given nucleotide sequence using Neighborhood-Joining Method (with bootstrap)

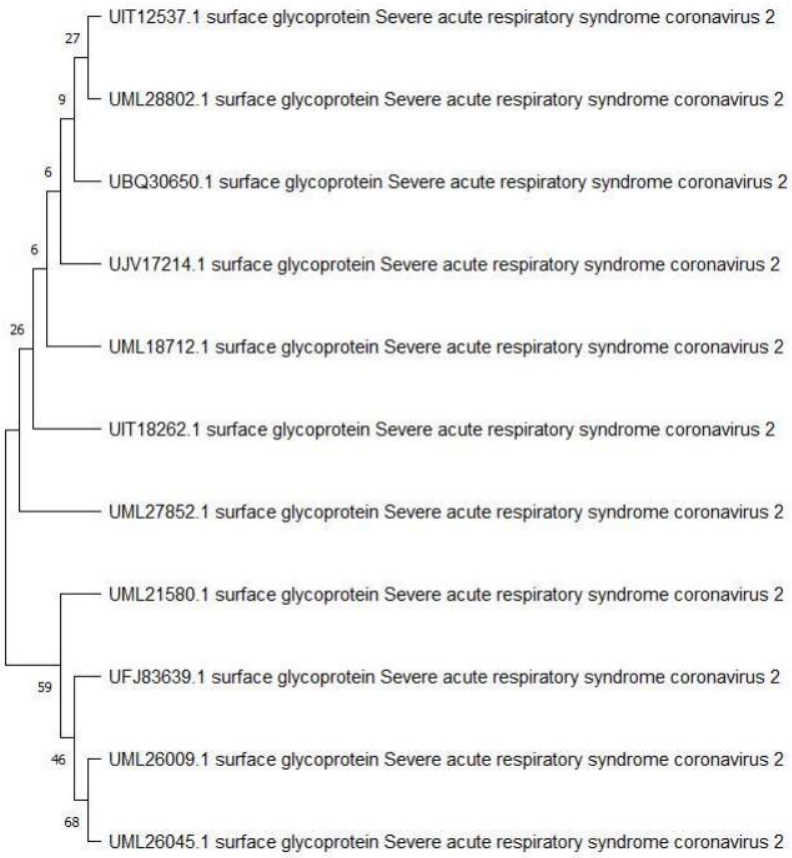

Fig: Phylogenetic Tree of given nucleotide sequence using Maximum-Likelihood (ML) method

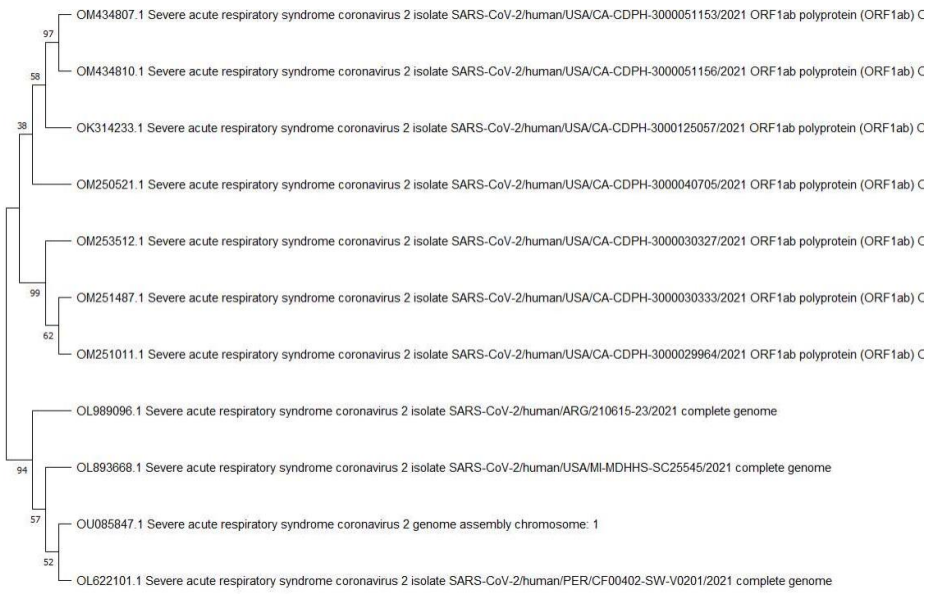

Fig: Phylogenetic Tree of given nucleotide sequence using Maximum-Likelihood (ML) method (with bootstrap)

MP, NJ, ML Trees (with 500 bootstrap) for protein multiple sequence alignment

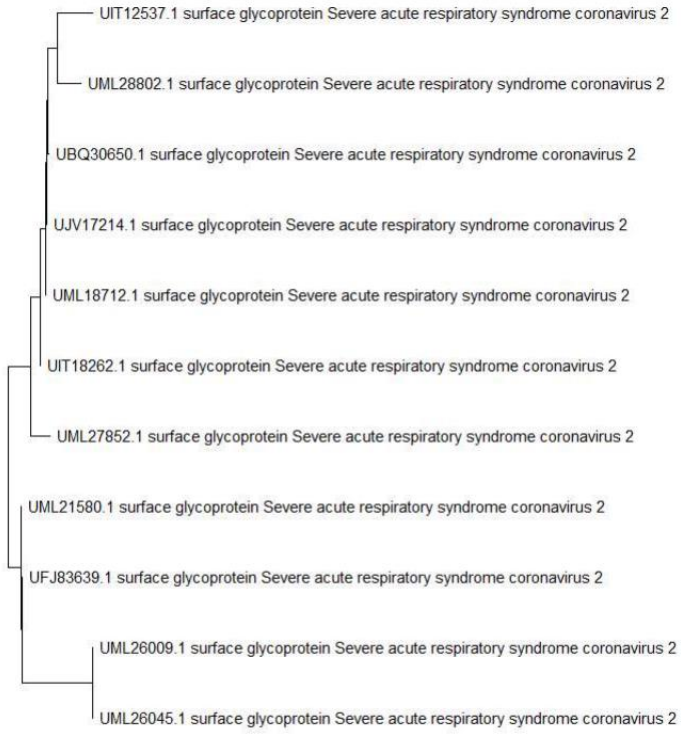

Fig: Phylogenetic Tree of given protein sequence using Maximum-Parsimony (MP) method

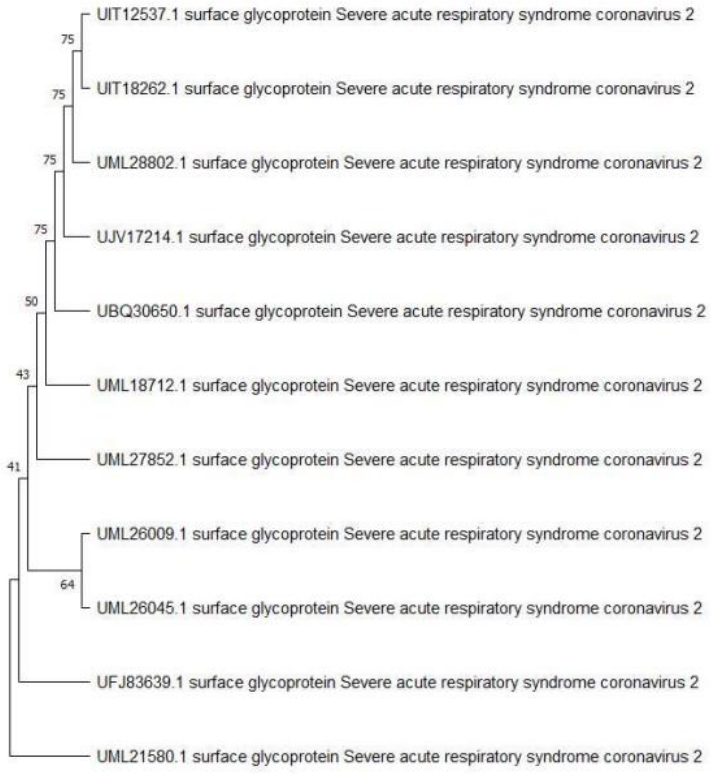

Fig: Phylogenetic Tree of given protein sequence using Maximum-Parsimony (MP) method (with bootstrap)

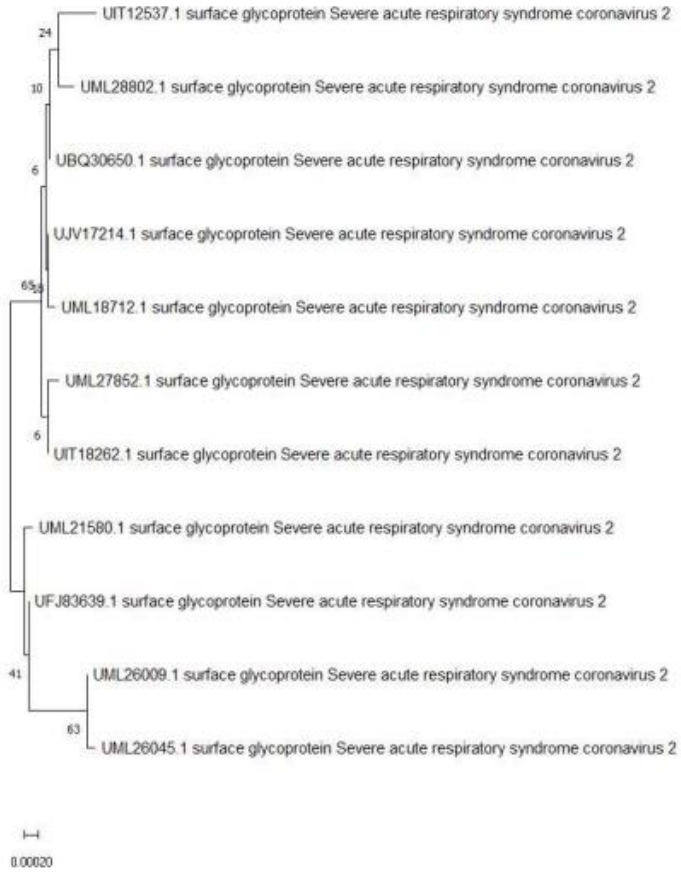

Fig: Phylogenetic Tree of given set of protein sequence using Neighborhood Joining (NJ) method

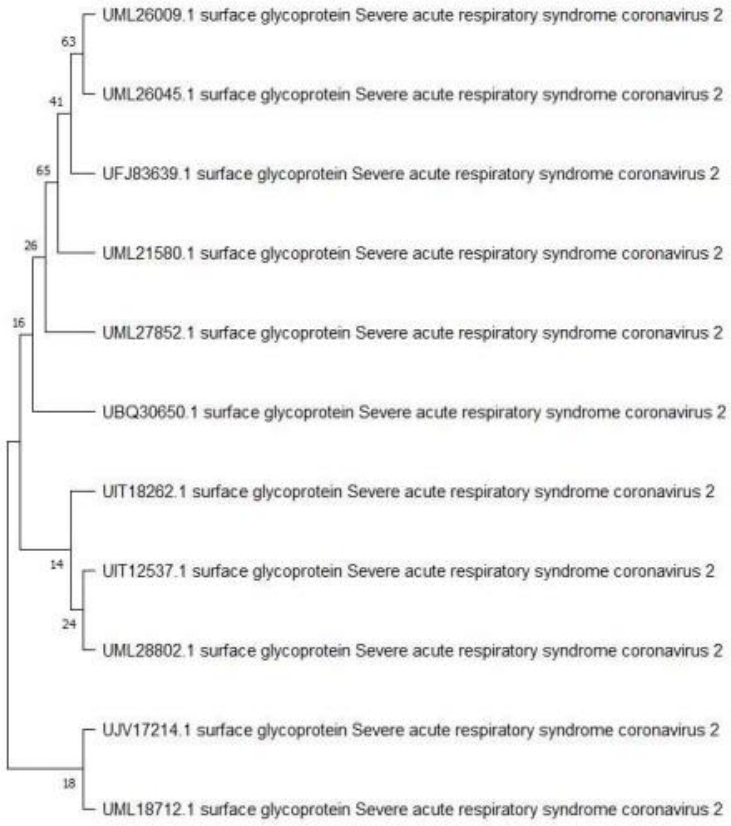

Fig: Phylogenetic Tree of given set of protein sequence using Neighborhood Joining (NJ) method (with bootstrap)

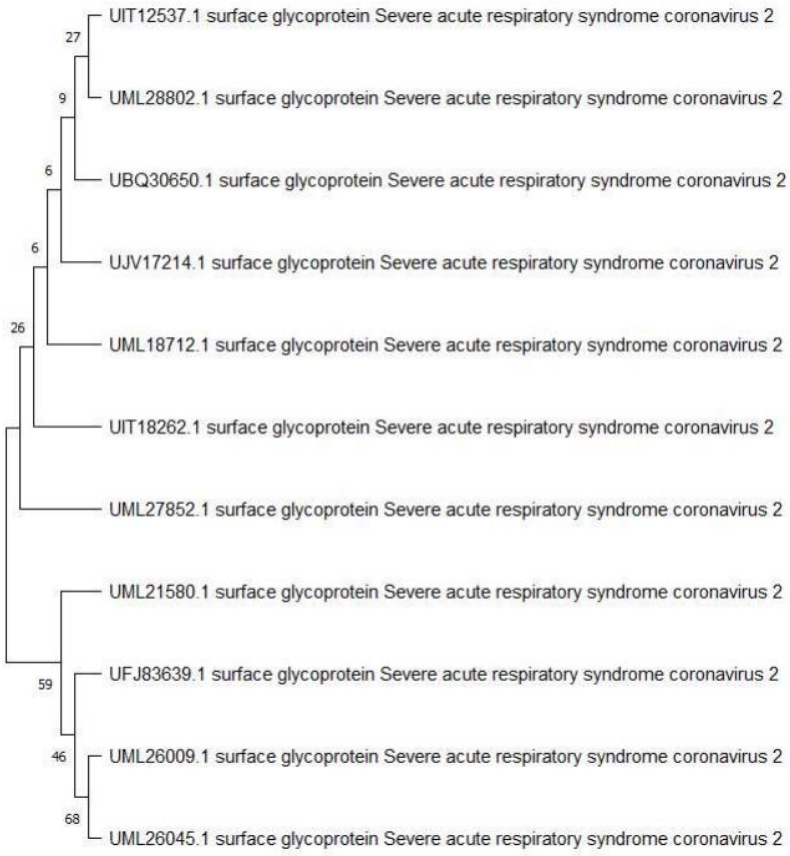

Fig: Phylogenetic Tree of given set of protein sequence using Maximum Likelihood (ML) method

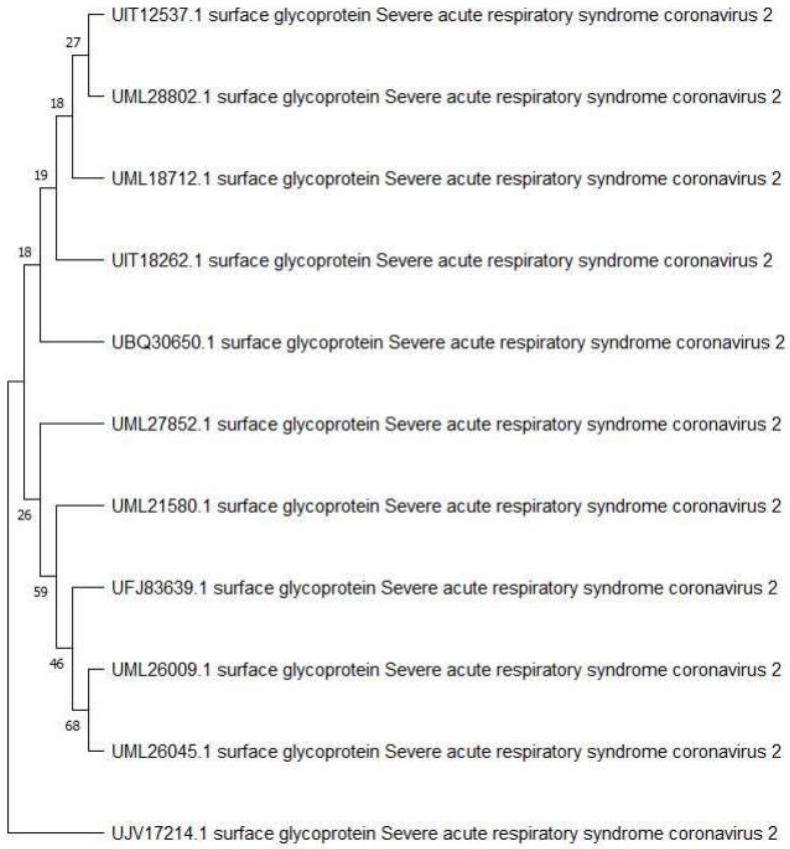

Fig: Phylogenetic Tree of given set of protein sequence using Maximum Likelihood (ML) method with bootstrap

Conclusion/Inference

As we know, in the maximum parsimony method, we choose trees minimizing the number of changes required to explain the data. Here, since the sequences are highly similar as shown by the BlastN, BlastP and Needle analysis, the maximum parsimony method can be used to construct the phylogenetic tree. Distance-based methods such as Neighbourhood Joining method build trees using evolutionary distances between operational taxonomic units. In Maximum Likelihood method, under a model of sequence evolution, we find a tree which gives highest likelihood of the observed data

1. <u>For nucleotide:</u>

   The Phylogenetic analysis of nucleotide sequences of Surface Glycoprotein UIT12537.1 (OM250521.1) using distance and character based methods shows that the most recent common ancestor (MRCA) is genes with accession number OM251487.1 and OM253512.1 along with OM251011.1 with a Confidence value of 99.

2. For Protein:

   The Phylogenetic analysis of protein sequences of Surface Glycoprotein UIT12537.1 (OM250521.1) using Maximum Parsimony shows that the most recent common ancestor (MRCA) are Surface Glycoprotein UIT12537.1 and Surface Glycoprotein UIT18262.1 with a Confidence value of 75.

   The Phylogenetic analysis of protein sequences of Surface Glycoprotein UIT12537.1 (OM250521.1) using Neighbourhood Joining shows that the most recent common ancestor (MRCA) are Surface Glycoprotein UML26009.1 and Surface Glycoprotein UML26045.1 with a Confidence value of 63.

   The Phylogenetic analysis of protein sequences of Surface Glycoprotein UIT12537.1 (OM250521.1) using Maximum Likelihood shows that the most recent common ancestor (MRCA) are Surface Glycoprotein UIT12537.1 and Surface Glycoprotein UML28802.1 with a confidence value of 27, along with Surface Glycoprotein UML26009.1 and Surface Glycoprotein UML26045.1 with a confidence value of 68.

References:

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4. **https://www.ncbi.nlm.nih.gov/blast/**
5. **https://www.ebi.ac.uk/Tools/msa/clustalo/**
6. **https://www.ebi.ac.uk/Tools/psa/emboss_needle/**
7. **https://www.megasoftware.net/**
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10. oup=gfind) respectively and analyze the given gene sequence
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