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Technical Note

Bioinformatic analysis reveals that some bacteria may aid SARS–CoV-2 spread and entry into host cells

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ABSTRACT

It is known that there are direct and indirect interactions between bacteria and viruses. Like some other viruses, we hypothesized that SARS-CoV-2 (Covid-19) may induce bacteria growth to aid its proliferation or may cooperate with bacteria to facilitate its activities in host cells. To verify this hypothesis, some bioinformatic tools and databases were employed. Complete genome sequence of SARS-CoV-2 was used to predict the bacteria with the required features. Results reveal that > 2000 bacteria were found to possess the required features using SMART tool. In addition, we compared different genome sequence of ACE-2 (belonging to different species) with some bacteria. Surprisingly, some bacteria were predicted and many predicted proteins are obtained. Finally, we compared some sequences of SARS-CoV-2 spike proteins with the predicted bacteria using BLAST tool. Results reveal many predicted proteins. These possible connections and similarities may be important for the virus enter the host cell. Until the virus finds the appropriate ACE-2 receptor to enter the host cell, bacteria may support the virus in this process. Perhaps even a virus attached to the bacterial surface which may be predicted to be a carrier for inter-host transmission. With the experimental demonstration of these hypotheses, the way to obtain new data on the reasons for the reproduction of the virus in the human body and the easy transmission of the virus in the population will be opens up. More than 10 different epitopes are found with signal peptide Therefore, focusing on bacteria-virus interactions opens up the window to exploit new future therapeutic targets.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 is a novel zoonotic virus that is transmitted from animals to humans, causing severe respiratory disease. It can be also be transmitted between humans. In this regard, it has recorded a fast and huge spread almost in all continents of the world, resulting in a pandemic [1, 2]. Recorded cases are more than 96.267.473 and the number of death has exceeded 1.940.352 [https://covid19.who.int/ 13 Ocak 2020]. Dysfunction of organs including lungs and gastrointestinal tract [3], abnormal liver function including splenic atrophy and lymphadenopathy are among the symptoms of SARS-CoV-2 [4, 5]. SARS-COV-2 has spike proteins which has two functional subunits (S1, S2). The S1 subunit plays a role during binding to the ACE-2 (Angiotensin-converting enzyme 2) receptor on the host cell, which is a crucial step for the virus entry into epithelial cells. On the other hand, the S2 subunit is an important fusion protein [6–9].

There are two types of bacterial-virus interactions; (a) direct interactions that in some way aid the virus, and (b) indirect interactions that aid the bacteria. Mechanisms supporting these interactions have been identified as (i) virus-induced increase in bacterial cell receptors; (ii) viral damage to the underlying epithelial cells; (iii) viral displacement of the commensal bacteria; and (iv) viral suppression of the host immune system [10]. Although the number of this interaction differ in organs, commensal bacteria ranging from 200 species (within the oral cavity) to 1000 species at the distal intestine (10^14 cells/g) occupy different organs including the gastrointestinal tract. Enteric viruses encounter these largely diverse commensal bacteria, and exploit them to facilitate the disease process. As an example, gastrointestinal microbiota has been shown to not only increase poliovirus infectivity, but may also promote host-host viral transfer. This situation resulted to certain symptoms such as chronic lung disease and pneumonia [11–14]. A huge number of commensal and pathogenic organisms colonize the nasopharynx, causing infections in the lower and upper respiratory tract, especially when the host immune system is compromised. For Covid-19, there are several evidences of dysfunction of organs including the gastrointestinal tract [14–16]. Approximately 29% to 39.2% of SARS-CoV-2 patients experience diarrhea and other digestive tract symptoms, with these symptoms occurring ~3.5–7.5 days after the onset of fever. This is the likely period for the virus to infect the immune cells, and then circulate to attack and damage the gut. Host immune response is very crucial in the fight against viruses [17–19].

An in vitro study demonstrated that viruses maybe able to directly bind to their target host cell and undergo replication with ease. However, this strategy may turn problematic in the gastrointestinal tract where a large number of bacteria occupy tissue surfaces, and directly competes for receptor binding sites thereby reducing the likelihood of bacterial proliferation or viral attachment. During attachment, some viruses may utilize bacterial ligands to enhance their association with the host cells, initiating the process of infection. This same strategy may be employed by some viruses that may not exclusively target the host's epithelial cells but may utilize the bacteria to facilitate the infection of other cell types. Moreover, some viruses may target cells such as lymphocytes, macrophages and monocytes [20–22]. Viruses consume alveolar macrophages and disrupt the bacterial clearance of pathogenic *S. pneumonia*. Studies have also documented the promotion of such dynamics. In addition, viruses alter the toll-like receptor pathways, causing a decrease in neutrophil extraction, which consequently increase the attachment of bacterial cells to the host epithelium. For instance, influenza infections have been found to make their host more susceptible to bacterial infection [23]. Therefore, this suggests the possibility that bacterial may aid viral infection via promotion of the viral disease symptoms. Studies have shown that poliovirus replicates in the intestine before it spreads and causes disease in the host. More also, a comprehensive study revealed that bacteria helps virus to bind more effectively to the host, and this was exemplified by poliovirus–bacteria interaction, which increased the viral titers in the host up to 500% [24, 25]. The reason for this is that bacteria components increased the binding of the virus to the host cell's receptor. Also, the host cell receptors for human norovirus have been reported to have histo-blood group antigens (HBGA)-like portions on the surface of some enteric bacteria (such as Enterobactercloacae). Poliovirus and norovirus are good examples of viruses whose pathogenesis is enhanced by direct binding of commensal enteric bacteria. It has been demonstrated that the presence of bacteria enhance viruses to infect or bind cells. Interestingly, a study demonstrated that bacterial synthesized enzymes can stimulate viral infection. In this case, the presence of the bacteria did not only increase viral adhesion, but also enhanced the infectivity of the virus. Another example is human immunodeficiency virus -*Mycobacterium tuberculosis* interaction accelerates the progression to acquired immune deficiency syndrome (AIDS)[26]. We have justified our hypothesis about the possibility of an interaction between SARS-CoV-2 and some bacterial species. We further hypothesize that SARS-CoV-2 may be affected by some bacterial species [14].

MATERIALS AND METHODS

Complete genome sequence of SARS-CoV-2 was obtained from NCBI SARS-CoV-2 database (Complete genomes: MN908947.3, MW505982, MW273795). Different sequences of ACE-2 such as NP_068576.1 (homo sapiens), BAB40370(human), P08473.2(human), BC048094.1(homo sapiens),AAH48094.2(homosapiens),NP_001034545.1(felis catus), BAD99266.1(homo sapiens), P12821.1(human),

ABN80106.1 (Mus Musculus), AAW78017.1(Rattus norvegicus) and XP_005169417.1(Danio rerio) were obtained from NCBI database. GenBank was used to obtain the taxonomy of the bacteria including Enterobacteriales (taxid: 91347), Firmicutes (taxid: 1239), Proteobacteria (taxid: 1224) and Actinobacteria (taxid: 201174)[27]. (https://blast.ncbi.nlm.nih.gov). Online SMART (smart.embl-heidelberg.de) tool was used to predict the similarity with the host proteome using the genome of SARS–CoV-2 [28]. SARS–CoV-2 genome sequence was divided into parts using online Pfam 32.0 (online:pfam.xfam.org) [29] and was blasted with BlastP tool and SMART tool. The physico-chemical properties of the proteins of interest were predicted using Expasy ProtParam (expasy.org/protparam) online server [30]. In addition, prediction of solubility was performed by SolPro. Phyre2 (Protein Homology/analogY Recognition Engine) was used to predict the structure of the protein of interest [31]. RasMol online bioinformatics tool was used to create the protein structure [32]. Signal peptides were analyzed with Signal-BLAST [33].

RESULTS AND DISCUSSION

The complete sequences of SARS–CoV-2 was obtained from NCBI, whole sequence's accession numbers are MN908947.3(Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome), MW505982 (Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/FRA/66JQ-O/2020, complete genome.), MW273795(Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/POL/PL_MCB_13/2020, complete genome). SMART tool was used to obtain all parts of SARS–CoV-2. These parts are already studied and they belongs to SARS–CoV-2 non-structural

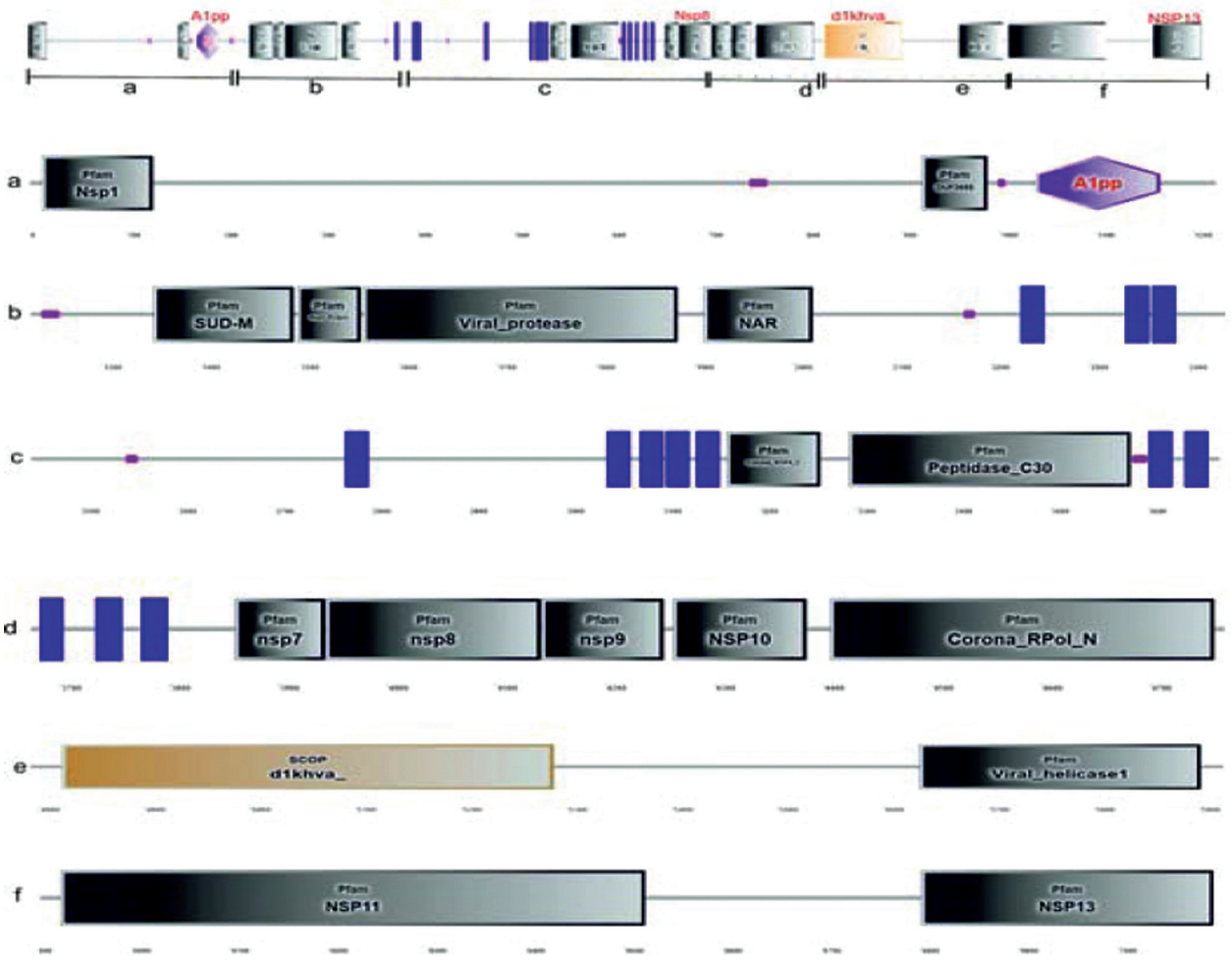


Figure 1. SMART tool are used to obtain this figure. SMART tool work with Pfam, Scop database. By using SMART tool, the parts were predicted irrespective of the different domains (whole sequence acc. num: MN908947.3, MW505982, MW273795) based on the whole genom of the SARS–CoV-2. Pfam domains (black), SMART domain (purple), SCOP domain (orange), the transmembrane proteins of SARS–COV-2 (colored blue in rectangulars).

proteins (Nsp) such as Nsp13 (Helicase), Nsp12 (RdRp), Nsp5 (3CLpro) and Nsp3 (PLpro) and structural protein[34]. We used MN908947.3, MW505982, MW273795 of SARS–CoV-2 and the same results were obtained for all. The same results are given on Figure 1.

Figure was obtained by SMART tool based on the sequences of the SARS-CoV-2 and the proteins in the databases have been brought together. Especially these results were taken in SMART, Pfam, Scop databases (are shown on figure 1). The first row of the figure is the original (a–f). Later these row were shown separately (a, b, c, d, e, f). One of the facilities provided by the SMART tool is that it easily shows the places to be looked at. SMART tool easly helped to connect with NCBI database to blast and compare the bacteria of interest with any part of SARS-CoV-2 sequence.

The groups of bacteria (Table 1) to be studied were estimated based on their protein domain which all existing protein domains table was created accordingly by using SMART tool. This bioinformatics tool predicted a massive number of bacteria based on the complete sequence of the SARS-COV-2. We decided to study with Actinobacteria, Firmicutes and Proteobacteria not only because they were predicted, but because these bacteria are abundant in the human body, and studies have shown that they are effective in spreading viruses [10–11, 14]. We found >2000 bacteria based on the complete sequence of SARS–CoV-2 (Table 2) by using SMART tool. The results are not only based on conserved region but also other proteins. Using BlastP, sequences of SARS-CoV-2 and that of some bacteria types were compared. The bacteria were Enterobacteriales (taxid: 91347), Firmicutes (taxid: 1239), Proteobacteria (taxid: 1224) and Actinobacteria (taxid: 201174). During blasting with NCBI, ref-seq protein were preferred so as to obtain more reliable results (Table 3). As expected, no similarity was found between all the parts of SARS-CoV-2 sequence and bacteria sequence based on SARS-CoV-2 FASTA form. On the other hand, one of these SARS-CoV-2 sequence parts, known as A1pp, was estimated to be similar to a of bacteria. However, no similarity was found for the others parts of SARS-CoV-2 which are DUF3655, SUD-M, Nsp3-PL2pro, Viral protease, NAR and NSP7. We investigated these parts using two different places in BlastP (ref-seg protein and non-redundant protein sequences) in order to ensure reliability of the results (Figure 1a-f). For both ref-seg protein and non-redundant protein sequences was no result in either place. However, of all other parts (Table 2) of SARS CoV parts predicted the results obtained, E.coli was the most predicted bacteria.

It is clear known that many of bacteria are belongs to our gut microbiota [10, 11](Table 1). This microbiota play

Table 1. More than 2000 bacteria were found based on based on the whole genome of SARS-CoV-2 (whole sequence acc. num: MN908947.3, MW505982, MW273795). All these are bacteria estimated based on their protein domain by using SMART tool and BlastP

Bacteria Family	Name	
Archaea (106)	Crenarchaeota (32)	
Undefined Kingdom (106)	Candidatus korarchaeota (1)	
	Euryarchaeota (73)	
Bacteria (2209)	Acidobacteria (9)	Fibrobacteres (2)
Undefined Kingdom (2209)	**Actinobacteria (527)**	**Firmicutes (691)**
	Aquificae (14)	Fusobacteria (21)
	Armatimonadetes (3)	Ignavibacteriae (3)
	Bacteroidetes (97)	Nitrospirae (2)
	Balneolaeota (1)	Planctomycetes (15)
	Caldiserica (1)	**Proteobacteria (582)**
	Calditrichaeota (2)	Spirochaetes (38)
	Chlamydiae (22)	Synergistetes (17)
	Chlorobi (2)	Tenericutes (2)
	Chloroflexi (14)	Thermodesulfobacteria (7)
	Coprothermobacterota (3)	Thermotogae (17)
	Cyanobacteria (50)	Verrucomicrobia (9)
	Deferribacteres (5)	Candidatus (2)
	Deinococcus (42)	
	Dictyoglomi (2)	

Table 2. After blasting bacteria which may similar to some parts of the virus predicted non-structural proteins of SARS-CoV-2 are shown. Common proteins between SARS-CoV-2 and some bacteria were found. The results were obtained by using SMART and BlastP, one after the other, based on their taxid

Name of parts	Types of bacteria
NSP1	CFB group bacteria
DUF3655	No Any Tpes Of Bacteria
A1pp	Thermotogales, Crenarchaeotes, Stony corals, Proteobacteria, GNS Bacteria (Bacteria:Caldisericumexile), Eukaryotes, D-Proteobacteria, Oomycetes, Firmicutes, Clostridium
SUD-M	No Any Tpes Of Bacteria
Nsp3-PL2pro	No Any Tpes Of Bacteria
Viral protease	No Any Tpes Of Bacteria
NAR	No Any Tpes Of Bacteria
Corona NSP4-C	*Escherichia coli*
Peptidase C30	*Escherichia coli*
NSP7	No Any Tpes Of Bacteria
NSP8	*Escherichia coli,* Proteobacteria
NSP9	Proteobacteria
NSP10	*Escherichia coli*, Proteobacteria (a, g)
corona Rpol-N	*Escherichia coli*
NSP13	*Escherichia coli*
NSP11	*Escherichia coli*
Viral-helicase1	*Escherichia coli, Rhizobium sullae, Rhizobiales bacterium*, Leisingera sp.
D1khva_	*Escherichia coli, Afipia broomeae*, Bradyrhizobium

a vital role for our immune system and it causes some diseases. Moreover, it is very important our healty balance, play role for protecting some diseases. For our hypothesis, having *E. coli* in the results can be considered positive. This is because we previously intended to focus on *E. coli.* Moreover, it is known that E.coli plays a vital role in the immune system by contributing to production of vitamins in the intestine. There is a massive population of *E. coli* in the human intestine [35].

We predicted similar features with these sequences which are MN908947.3, MW505982, MW273795, that is, the connectivity of ACE-2 to bacteria in different species, moreover proteins similar to both spike protein (MN908947.3, MW505982, MW273795 were used) and ACE-2 were found in bacteria by using BlastP.

The predicted results found were based on the special regions. Regions similar to the regions where SARS-CoV-2 binds to ACE2 were also found within bacteria In particular, regions where the bacteria bind to the SARS-CoV-2 spike protein were used. And then compare the sequences of ACE-2 belonging to some species (ranging from Homo sapiens to Danio rerio) with bacteria were compared. Their accession numbers are NP_068576.1(Homo sapiens), BAB40370(Homo sapiens), P08473.2(Homo sapiens), BC048094.1(Homo sapiens), AAH48094.2(), NP_001034545.1(Felis catus), BAD99266.1(Homo sapiens), P12821.1(Homo sapiens), ABN80106.1(Mus musculus), AAW78017.1(Rattus norvegicus) and XP_005169417.1(Danio rerio). We are found interaction between the bacteria and ACE-2 by using results of Table 1, it will positively validate our hypothesis. It is important, since SARS-CoV-2 binds to ACE-2 receptor to enter the host cell, the virus can intereact or bind (for a short period) with bacteria which has similar structer surface receptor to ACE-2. According to the results of the Smart bioinformatics tool, the similarities of suitable bacterial types were examined To find the similarity, we compared the sequence ACE-2 with some of the predicted bacteria using BlastP. The bacteria sharing common characteristics with the different sequences of ACE-2 are shown in Table 3.

Different numbers of bacteria were estimated in each of the bacteria species whic are Enterobacteriales (taxid: 91347), Firmicutes (taxid: 1239), Proteobacteria (taxid: 1224) and Actinobacteria (taxid: 201174). Some of these bacteria have been found to be abundant in the human body (Table 1) [10–11]. Another feature of Table 4, and most important, is the similarity between the spike protein sequences (MN908947.3, MW505982, MW273795)) of SARS-CoV-2 and some bacterial proteins that were found after blasting with SMART tool (Table 3). Based Enterobacteriales (taxid: 91347), Firmicutes (taxid: 1239),

Proteobacteria (taxid: 1224) and Actinobacteria (taxid: 201174) which predicted by their protein domains, we used tools that are SMART, Pfam, Phyre 2 and BlastP to predicted proteins but similar on ACE-2 receptor. The result shows the prediction of some similarities between spike protein sequence and the bacterial protein (predicted protein and shown Table 4). Low similarity means low probability of binding. If there is a predicted connection between the SARS-CoV-2 spike protein and the bacterial protein are predicted on Table 4 (Hypothetical proteins, Helix-turn-helix transcriptional regulator, extracellular solute-binding protein, YhgE/Pip domain-containing protein), it can be considered as a short-term binding of the bacteria with using the ACE-2. This implies that SARS–CoV-2 may be attached to the bacteria for a short time. When SARS-CoV-2 binds ACE-2 receptor, which has stronger common features with the SARS-CoV-2 based on our prediction, it can lose its interaction with bacteria. The possibility that the virus has a short-term attachment to the bacteria also supports the view that the bacteria help the virus enter the host.

We obtained many more than 20 different types of Hypothetical protein(ACE-2-like proteins of bacteria) which the spike protein of the virus may interact with. but we used some of them (shown Table 4). The results for *Escherichia coli* are WP_077697961.1, WP_162751851.1, WP_148724040.1 respectively. Also, Firmicutes (especially Bacillus, Clostridium) and Actinobacteria were used to

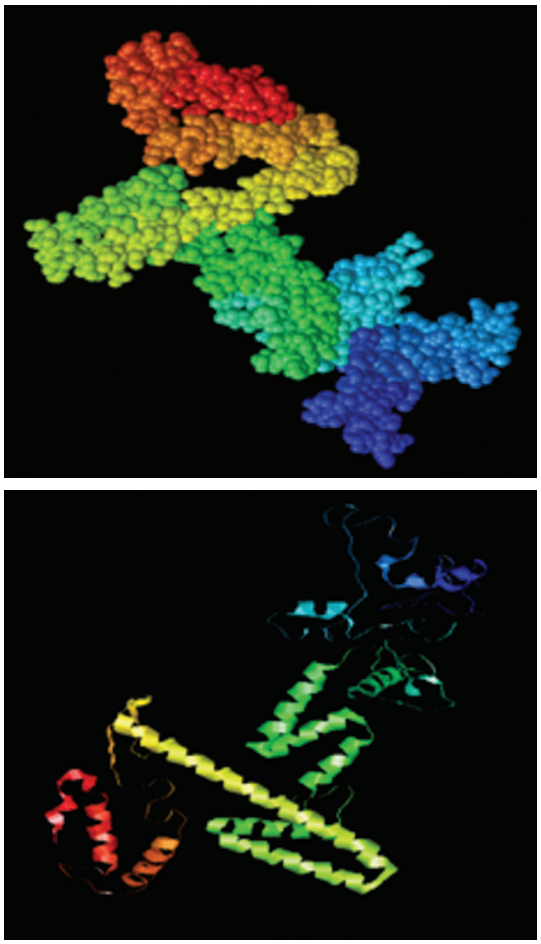
Table 3. BlastP were used compares the sequence of ACE-2 with the bacteria groups. According to the blasting result, some bacteria were found to have proteins similar to the ACE2 receptor. Different whole ACE-2 are used NP_068576.1(Homo sapiens), BAB40370(Homo sapiens), P08473.2(Homo sapiens), BC048094.1(Homo sapiens), AAH48094.2(), NP_001034545.1(Felis catus), BAD99266.1(Homo sapiens), P12821.1(Homo sapiens), ABN80106.1(Mus musculus), AAW78017.1(Rattus norvegicus) and XP_005169417.1(Danio rerio). Blasting was done separately for each bacterial species. These different types ACE-2 were chosen because they were close to humans in the phylogenetic similarity

Acc. number of ACE-2	Name of bacteria
BAB40370	Enterobacteria, (*E.coli, Klebsiella aerogenes, Proteus mirabilis*), Proteobacteria, Bacillus, *Novibacillus thermophilus*
BC048094.1	
AAH48094.2	
NP_001034545.1	Proteobacteria, Enterobacteria (*E.coli*), Firmicutes (Bacillus) Actinobacteria (Mycobacterium)
BAD99266.1	CFB group bacteria, Proteobacteria, Firmicutes, some Rhodopirellula and Planctomycetes, Actinobactria
NP_068576.1	
P08473.2	Firmicutes (more than 90 hits), *E.coli, Klebsiella aerogenes, Providencia stuartii,* Actinobacteria
ABN80106.1	Actinobacteria (Mycobacterium), Enterobacteria (*E.coli*), Proteobacteri
AAW78017.1	
XP_005169417.1	Actinobacteria, Enterobacteria, Proteobacteri

Table 4. The results obtained from the BlastP tool (using refseg-protein). These results were obtained by processing the bacteria, one afar the other, based on their taxid. The presence of these proteinsbelonging to bacteria may increase the probability of possible binding of spike proteins. Because these results are proteins that the spike protein of the virus may interact with. Besides, shown here are ACE-2-like proteins of bacteria (*Streptomyces lincolnensis, Roseburia inulinivorans, Escherichia coli,* Klebsiella)

Description	Ident	Accession
Streptomyces lincolnensis, -extracellular solute-binding protein	32.53%	WP_067433226.1
Roseburia inulinivorans -YhgE/Pip domain-containing protein	37.70%	WP_007884493.1
Escherichia coli -Hypothetical protein	35.10%	WP_077697961.1
Escherichia coli -Hypothetical protein	35.00%	WP_162751851.1
Helix-turn-helix transcriptional regulator [Klebsiella]	34.21%	WP_045783066.1
Escherichia coli -Hypothetical protein	26.10%	WP_148724040.1

Table 5. WP_077697961.1 is obtained *Escherichia coli* -Hypothetical protein(ACE-2-like proteins) are obtained by using both Phyre2 online tool and RasMol. Signal-BLAST, Expasy ProtParam are used to the physical and chemical properties. Even more than 30 B-cell epitopes were found, Value of B-cell epitopes more than 0.75 were shown. This *Escherichia coli* -Hypothetical protein may play a role to carry virus to host. After signal blasting, signal peptide is predicted strong. These table are close for other *Escherichia coli* -Hypothetical proteins which are WP_148724040.1 and WP_162751851.1 are shown Table 4, they were not shown due to not to so different results (are shown Table 5)

Description : WP_077697961.1	**The prediction results**
	Non transmembrane protein 0.793405 Alpha helical transmembrane protein 0.0887929 Beta barrel transembrane protein 0.117802 Predicted domains: 1-97, 98-371 Does have disulfide bonds. Soluble with probability 0.777470 Antigenicity: 0.5856006 Capsid sequence: NO (distance = –1.291755) Tail Sequence: NO (distance = –1.019186) Formula: $C_{1830}H_{2777}N_{463}O_{526}S_{30}$ Molecular weight: 40641.77 Theoretical pI: 4.88 Aliphatic index: 87.52 Gravy: 0.236 Signal Peptide: strong Most Likely Epitopes: 0.8797327 229 GQTAKQD 0.84221798 229 GQTAKQ 0.83097992 161 SRSIED 0.81062069 122 IPTNFV 0.80333053 228 AGQTAK 0.7940375 228 AGQTAKQ 0.78473436 310 PITNTV 0.77244283 229 GQTAKQDV 0.76815851 300 CPGFNT

predict some hypothetical bacterial proteins(ACE-2-like proteins of bacteria) related to the spike proteins. These ACE-2-like proteins of bacteria are WP_067433226.1, WP_007884493.1, WP_045783066.1 (shown on Table 4). For all of these spike proteins, Actinobacteria, Enterobacteria and Firmicutes were predicted to may help the virus enter the host virus attached to the bacterial surface which may be predicted to be a carrier for inter-host transmission. Even we found domain-containing protein and extracellular solute-binding protein, we did not use them.

As a result of the use of Pfam, SMART, Phyre2, Expasy ProtParam, Signal-BLAST, RasMol bioinformatics tools, many features were obtained such as: molecular weight, theoretical pI, amino acid composition, atomic composition, instability index, aliphatic index and grand average of hydropathicity were given in the tables. The estimated formulas of the molecules are shown in all tables. We decided to document some important few of these features. Further, the bioinformatics tools were used to also predict the disulfide bonds, B-cell epitopes. These are two features commonly used in protein protein adhesion and vaccine studies. It is already known that the sulphate bond is very important in the structure of the protein. In addition, determination of epitope in vaccine studies is essential for the pre-candidate vaccine. The epitope values we found turned out to be quite good. These results are shown in the tables. Normally, if an epitope above 0.5 is sufficient, our values are around 0.8. [33, 36, 37]. All tables (Tables 5–8)

Table 6. WP_045783066.1 is obtain as *Klebsiella* Helix-turn-helix transcriptional regulator protein(ACE-2-like proteins) by using both Phyre2 online tool and RasMol. Signal-BLAST, Expasy ProtParam are used to the physical and chemical properties. More than 20 epitopes were estimated, six of them are used but signal peptides are not strong as Table 5

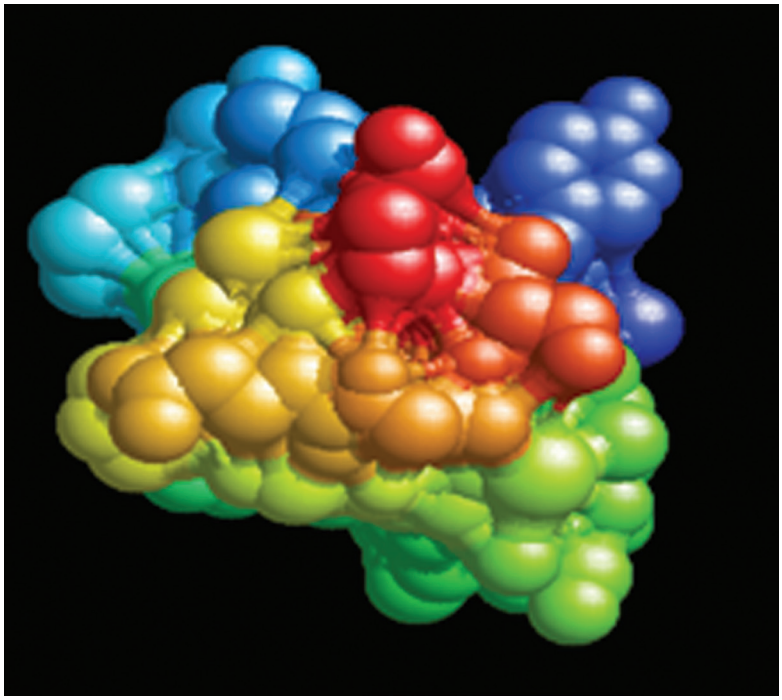
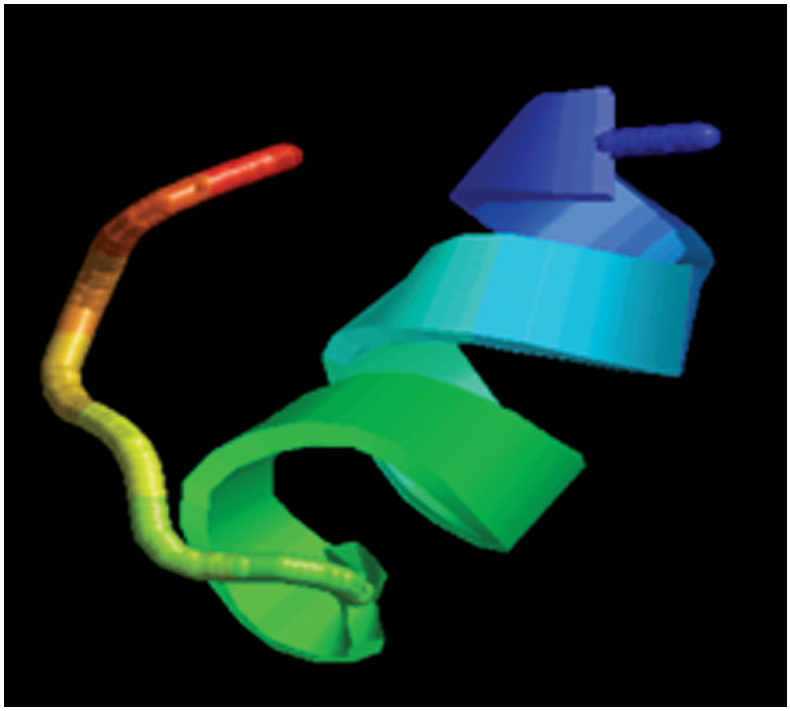
Description: WP_045783066.1	**The prediction results**
	Non transmembrane protein 0.851199 Alpha helical tansmembrane protein 0.137226 Beta barrel transembrane protein 0.0115744 Predicted domains: 1–20 cannot form disulfide bonds. Soluble with probability 0.818427 Antigenicity: 0.52493 Capsid sequence: NO (distance = –0.201569) Tail sequence: NO (distance = –0.938932) Formula: $C_{103}H_{149}N_{29}O_{28}$ Aliphatic index: 73.50 Gravy: –0.155 Molecular weight: 2431.83 Theoretical pI: 5.0998 Signal Peptides: weak Most Likely Epitopes: 0.7150423 10 RGSGWT 0.64396864 10 RGSGWTA 0.63795379 9 LRGSGWT 0.636777 0 HLRDSA 0.61616636 9 LRGSGW 0.60012377 0 HLRDS
	

Table 7. WP_007884493.1 is obtained as *Roseburia inulinivorans* -YhgE/Pip domain-containing protein(ACE-2-like proteins) by using both Phyre2 online tool and RasMol. Signal-BLAST, Expasy ProtParam are used to the physical and chemical properties too. Even more than 25 B-cell epitopes were found, Value of B-cell epitopes more than 0.75 were shown. This *Roseburia inulinivorans* -YhgE/Pip domain-containing protein may play a role to carry virus to host. After signal blasting, signal peptide is predicted not strong

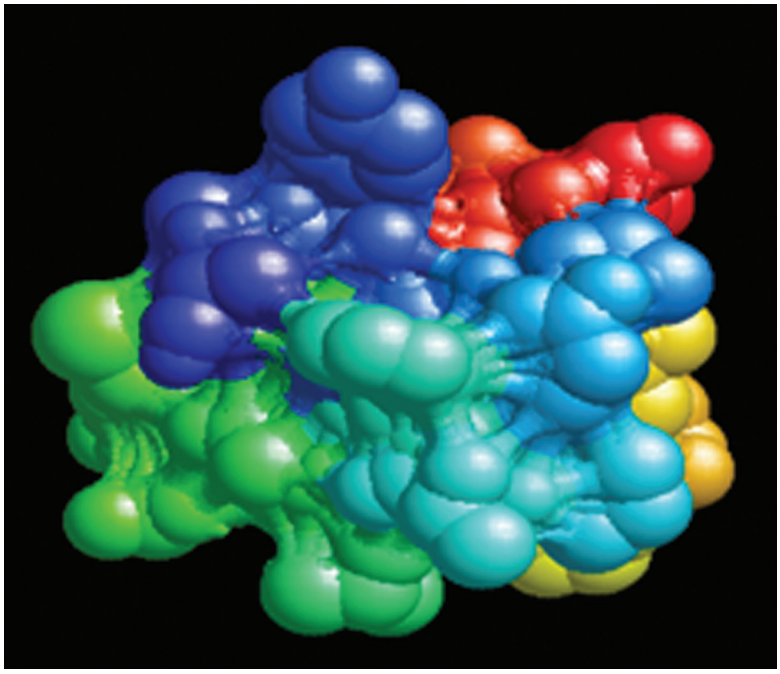
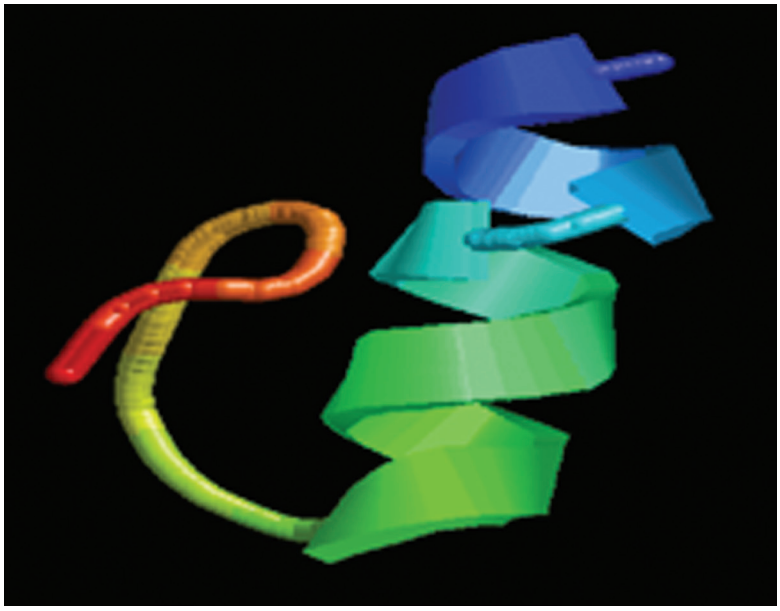
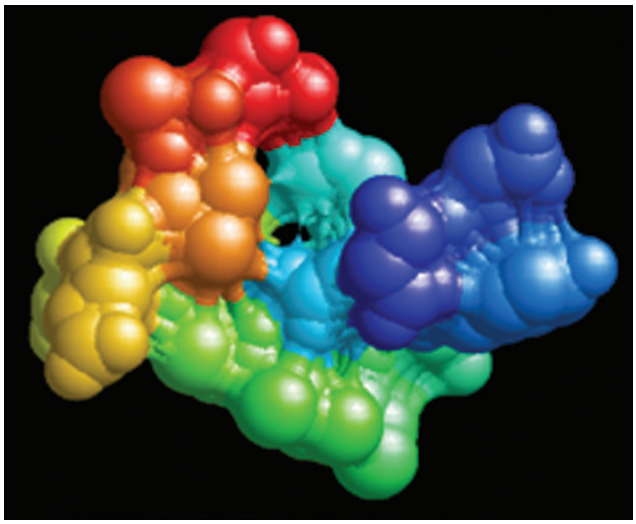
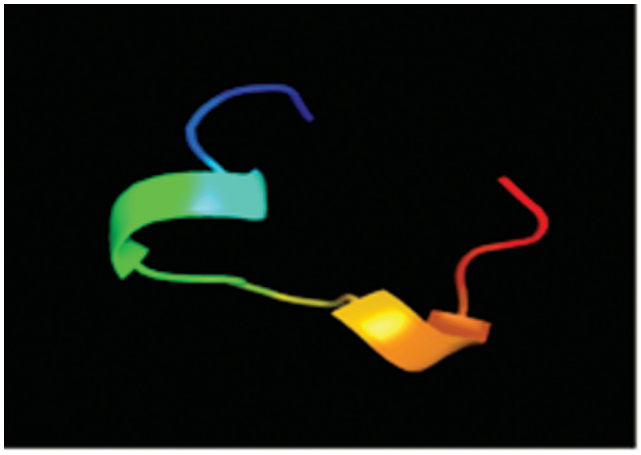
Description: WP_007884493.1	**The prediction results**
 	Non transmembrane protein 0.822297 Alpha helical tansmembrane protein 0.153377 Beta barrel transembrane protein 0.024326 Predicted domains: 1–35, cannot form disulfide bonds Soluble with probability 0.658794 Antigenicity: 0.77224 Capsid sequence: YES (distance = 0.020664) Tail sequence: NO (distance = –0.711737) Formula: $C_{112}H_{183}N_{27}O_{34}$ Aliphatic index: 135.65 Gravy: 0.739 Molecular weight: 2451.85 Theoretical pI: 5.84 Signal found (weak) Most Lıkely Epitopes: 0.93413642 1 QFSSAG 0.76262772 0 NQFSSAG 0.70368048 1 QFSSA 0.828431 0 NQFSSA 0.80841218 1 QFSSAGL 0.80548814 2 FSSAGLQ 0.79698949 2 FSSAGL

Table 8. WP_067433226.1 is obtained as *Streptomyces lincolnensis*, -extracellular solute-binding protein (ACE-2-like proteins) by using both Phyre2 online tool and RasMol. Signal-BLAST, Expasy ProtParam are used to the physical and chemical properties too. Even more than 15 B-cell epitopes were found, Value of B-cell epitopes more than 0.7 were shown. This *Streptomyces lincolnensis*, -extracellular solute-binding protein may play a role to carry virus to host. After signal blasting, signal peptide is predicted not strong

Description: WP_067433226.1	**The prediction results**
 	Non transmembrane protein 0.899067 Alpha helical transmembrane protein 0.0923786 Beta barrel transembrane protein 0.00855444 Predicted domains: 1–30 cannot form disulfide bonds Soluble with probability 0.948514 Antigenicity: 0.7538 Capsid sequence: NO (distance = –0.075400) Tail sequence: NO (distance = –0.704089) Formula: $C_{151}H_{209}N_{35}O_{48}$ Aliphatic index: 86.67 Gravy : –0.907 Molecular weight: 3282.53 Theoretical pI: 3.90 Signal Peptides: weak Most Lıkely Epitopes: 0.76765773 19 LDLGKY 0.76408068 20 DLGKYW 0.7399505 19 LDLGKYW 0.73250645 20 DLGKYWW 0.7251304 10 DIGENE 0.70512869 18 NLDLGKY

include the predicted results of proteins which are shown on Table 4. These results are both the physical and chemical properties. They are molecular weight, molecular formula, theoretical pI, amino acid composition, atomic composition, instability index, aliphatic index, non transmembrane protein, signal peptide, antigenicity and grand average of hydropathicity. All on tables showed that the predicted proteins have epitope regions. In addition, disulfide bonds were detected in Table 1. As mentioned earlier, more than 20 proteins (Hypothetical protein predicted to belongs to *Escherichia coli*) were detected and one complex of them are used only (Table 5).

CONCLUSION

Some similarities were predicted between SARS-CoV-2's spike protein and bacterial protein, bacteria and different sequences of ACE-2 (belonging to different species), as well as bacteria and some parts of SARS-CoV-2 by helping tools. Similarities between bacteria and different types of ACE-2's FASTA forms, amino acid forms were predicted) by using both Phyre2 online tool and RasMol. Signal-BLAST, Expasy ProtParam are used to the physical and chemical properties. This similarities between bacteria and different types of ACE-2 implies that SARS–CoV-2 may be attached to the bacteria for a short time. When SARS-CoV-2 binds

ACE-2 receptor, which has stronger common features with the SARS-CoV-2 based on our prediction, it can lose its interaction with bacteria. It is expected to separate from the bacteria, as strong interactions invalidate weak interactions. Some of protein, which were predicted to contain disulfide bonds, belong to the E.coli family of bacteria. These bonds may make the protein structures to be prone to chemical change so ıt may help to obtain different forms of our predicted results (are shown on Tables 5–8) [36]. We think that these possible connections and similarities may be important for the virus enter the host cell. Until the virus finds the appropriate ACE-2 receptor to enter the host cell, bacteria support the virus in this process. Perhaps even a virus attached to the bacterial surface which may be predicted to be a carrier for inter-host transmission. With the experimental demonstration of these hypotheses, the way to obtain new data on the reasons for the reproduction of the virus in the human body and the easy transmission of the virus in the population will be opens up. In conclusion, SARS-CoV-2 may influence bacteria to aid its proliferation. Therefore, the focus on bacteria–virus interactions opens up the window to exploit new future therapeutic targets.

DATA AVAILABILITY STATEMENT

No new data were created in this study. The published publication includes all graphics collected or developed during the study.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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