Revealing In Silico that Bacteria's Outer Membrane Proteins may Help our Bodies Replicate and Carry Severe Acute Respiratory Syndrome Coronavirus 2

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ABSTRACT: Some studies in the literature show that viruses can affect bacteria directly or indirectly, and viruses use their own specific ways to do these interactions. Furthermore, it is said that bacteria are prone to attachment mammalian cells during a viral illness using their surface proteins that bind to host extracellular matrix proteins such as fibronectin, fibrinogen, vitronectin, and elastin. A recent study identified the cooperation between bacteria and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in silico, in vitro, and in vivo. Like this study, we hypothesized that more bacteria protein might help SARS-CoV-2 transport and attach to angiotensin-converting enzyme 2 (ACE2). The bacteria's outer membrane proteins (OMPs) we chose were not random; they had to be on the outer surface of the bacteria because these proteins on the outer surface should have a high probability of interacting with both the spike protein and ACE2. We obtained by using bioinformatics tools that there may be binding between both ACE2 and spike protein of these bacteria's OMPs. Protein-protein interaction results also supported our hypothesis. Therefore, based on our predicted results, these bacteria OMPs may help SARS-CoV-2 move in our body, and both find and attach to ACE2. It is expected that these inferences obtained from the bioinformatics results may play a role in the SARS-CoV-2 virus reaching host cells. Thus, it may bring a different perspective to studies on how the virus can infect host cells.

KEYWORDS: Spike-ACE2 interaction, virus-bacteria interaction, COVID-19, SARS-CoV-2, bioinformatic

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Introduction

Coronavirus disease 2019 (COVID-19), caused by the new human pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been highly transmissible between people.[1] It has spread fast worldwide and is announced as a pandemic by the World Health Organization (WHO).[2,3] Severe acute respiratory syndrome coronavirus 2 has structure proteins which are spike (S), envelope (E), membrane (M), and nucleocapsid (N).[4] Spike protein plays a role diversity of SARS-CoV-2, while the other 4 structural proteins share almost 90% amino acid identity with SARS-CoV.[1,5] The spike protein has 2 functional subunits (S1 and S2). The S1 subunit (mutations were found) plays a vital role in binding to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell, crucial for the virus entry into epithelial cells. On the contrary, the S2 subunit is a vital fusion protein.[5-8] Bacteria and viruses interact in 2 ways: (1) viral binding to a bacterial cell or (2) viral utilization of a bacterial product. The direct interactions contain a specific bacterium or bacterial product that aids viral infection[9]—the cooperation between bacteria and viruses is quite interesting. Sometimes, viruses help bacteria spread, and sometimes bacteria help the virus replicate or spread in host cells.[9] There are some examples of direct interactions between bacteria and viruses. For example, the interaction between Human norovirus and *Enterobacter cloacae*. During this interaction, histo-blood group antigen (HBGA)-like moieties serve as co-factor during infection.[9-11] Also, Murine norovirus and *E. cloacae* have a direct interaction in which HBGA-like moieties act as co-factor during infection; evidence of the presence of intestinal microbiota aid establishment of persistent viral infection.[10,12] Another example of direct interaction between bacteria and viruses is a cooperation between Poliovirus and N-acetyl glucosamine–containing polysaccharides. This direct interaction enhanced cell association and viral replication; increased capsid stability and transmission.[13,14] Moreover, *Enteric bacteria* help Rotavirus by enhancing viral replication; enhancing virus binding/entry; and less-effective host antibody response.[15] *Mycobacterium tuberculosis* bacteria help the Human immunodeficiency virus (HIV) too. This bacteria increase HIV long terminal repeat-driven transcription and HIV production during this virus.[16] Like direct interaction, there is some indirect interaction between bacteria and viruses. For instance, *Streptococcus pneumoniae; Streptococcus aureus* increases host cell adhesion molecules during the Rhinovirus effect.[17] Like *S. pneumoniae* and Rhinovirus interaction, *S. pneumoniae* helps Adenovirus by increasing host cell adhesion molecules.[9,18] Another example is between Influenza virus—*S. pneumoniae; S. aureus; Haemophilus influenza* respiratory commensals. The logic behind this interaction is that viral neuraminidase cleaves epithelial cell sialic acid exposing bacterial receptors; damaging epithelial cells.[9,19,20]

Gastrointestinal (GI) habitats incorporate 200 species (within the oral cavity) to 1000 species at the distal intestine, where bacterial concentrations can get at almost 10^{14} cells/g.[21]

Table 1. Docking score of bacteria protein-ACE2 and bacteria protein-Spike RBD results predicted by HDOCK SERVER and ClusPro 2.0.

BACTERIA PROTEINS	RECEPTOR		
	HDOCK SERVER		CLUSPRO 2.0
	SPIKE RBD	ACE2	SPIKE RBD
PDB:1QU7	−287.79	−287.79	−1094,2
PDB:2ZFG	−285.78	−285.78	−1470
PDB:2XG6	−434.17	−305.41	−1763.2

Bacteria cell has top layers in the envelope. They are the outer membrane (OM), a distinguishing feature of Gram-negative bacteria, the peptidoglycan cell wall, and the cytoplasmic or inner membrane (IM). The outer leaflet of the OM is made of glycolipids, mainly lipopolysaccharide.[22] Outer membrane proteins (OMPs), such as the porins, OmpF, and OmpC, function to allow molecules such as mono disaccharides and amino acids across.[22] A class of OMPs, which are larger transmembrane β strands, but are present at much lower levels, function as gated channels in the high-affinity transport of large ligands.[23] Bacteria have OMPs that may cooperate with the virus.[9-20] Bacteria are prone to infect mammalian cells during a viral illness using their surface proteins that bind to host extracellular matrix proteins.[9,24,25] Even there is no significant clue to our knowledge whether bacterial infections in COVID-19 are directly attributable to SARS-CoV-2; we know that ACE2 receptors are related. The SARS-CoV-2 are expressed in a place where many species of bacteria occupy such as small intestine, duodenum, etc, and it is known that this virus may binds to cells in these places.[5,6,21,26,27] One of the severe clinical manifestations of COVID-19 is pneumonia and progression to acute respiratory distress syndrome (ARDS), especially in the elderly. Most of those intubated due to SARS-CoV-2 include these age groups, and their lungs are affected by SARS-CoV-2. When inflammation occurs in the lung, it can impact the gut microbiota. So there is a possibility that novel SARS-CoV-2 might also impact the gut microbiota.[28-32] It has also been stated that dysbiosis in the elderly appears to be linked to the severity of COVID-19.[33] It is known that there are both, direct and indirect, interactions between bacteria that occupy an enormous number in our body and viruses.[9-20,29-33] The purpose of this study is to pay attention to the possibility that proteins located outside the cell wall of bacteria which live with us and are located in our body may interact with the spike protein of SARS-CoV-2. These proteins may directly or indirectly affect the transmission and replication of the SARS-CoV-2 virus in the body.

Method

Outer membrane proteins of bacteria which are PDB:1QU7(4 helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor), PDB:2ZFG (Structure of OMPF porin), PDB:2XG6 (transport protein), and spike receptor-binding domain (RBD) structure (PDB:2GHV) were taken from Protein Data Bank (https://www.rcsb.org/)[34] to be used for examining the interaction between different SARS-CoV-2′ spike proteins. We used this OMP because we lived with bacteria with OMPs and located a massive number in our body.[21,22,29-31] Both HDOCK SERVER and ClusPro 2.0 tools were used for protein-protein docking (between spike proteins-bacteria proteins and ACE2 [Accession number: NP_068576.1 and sequenced in January 2021] and bacteria protein).[35,36] Two different protein-protein docking tools were used to check the consistency of the prediction, and both tools are highly used as docking tools and ranked as the best methods of critical assessment of the projection of interactions.[37,38] Phyre2 tool was used to obtain the tertiary structure of ACE2,[39] and the tertiary structure of ACE2 was used for HDOCK SERVER and ClusPro 2.0 to predict the docking score. All the structures are visualized using Chimera 1.15.[40]

Results and Discussion

Like interaction between Murine norovirus and *E. Cloacae*, Poliovirus and N-acetyl glucosamine–containing polysaccharides, *Enteric bacteria* and Rotavirus, *S. pneumoniae* and Adenovirus, Influenza virus and *S. pneumoniae, Mycobacterium tuberculosis* and Human immunodeficiency virus (HIV),[9-20] bacteria may play a role in the outbreak of coronavirus disease. Bacteria with OMPs live and locate a massive number in our body.[41] These bacteria OMPs may help SARS-CoV-2 to move and find its receptor. Therefore, this study, using bioinformatics tools, tried to make preliminary predictions that OMPs could play a role in the SARS-CoV-2 epidemic. For this purpose, some OMPs of bacteria (PDB:1QU7 [4 helical-bundle structure of the cytoplasmic domain of a serine chemotaxis receptor], PDB:2ZFG [Structure of OMPF porin], PDB:2XG6[transport protein]), were used. Moreover, spike RBD structure (PDB:2GHV) and ACE2 (Accession number: NP_068576.1) (The other 3 of them where there are no results are deleted) were exploited. All results are shown in Table 1 and Figures 1 to 3, respectively. Both HDOCK SERVER and ClusPro 2.0 tools were used for protein-protein docking (between spike protein and bacteria proteins and ACE2 [Accession number: NP_068576.1 and sequenced in January 2021] and bacteria protein)[35,36]

There is a massive amount of bacteria in the human body, and they play an essential role in maintaining host health by providing energy, nutrients, immunological protection, probiotic resistance, help virus interaction, and so on.[9-17,21,22] Although the relationship of bacteria with SARS-CoV-2 is not precisely known,[27] several studies have already been carried out to predict that there may be an interaction between bacteria and viruses.[9-20] Since it is known that it is not a long time for human beings and the SARS-CoV-2 to live together, there are many things not to understand precisely what helps virus replicate and

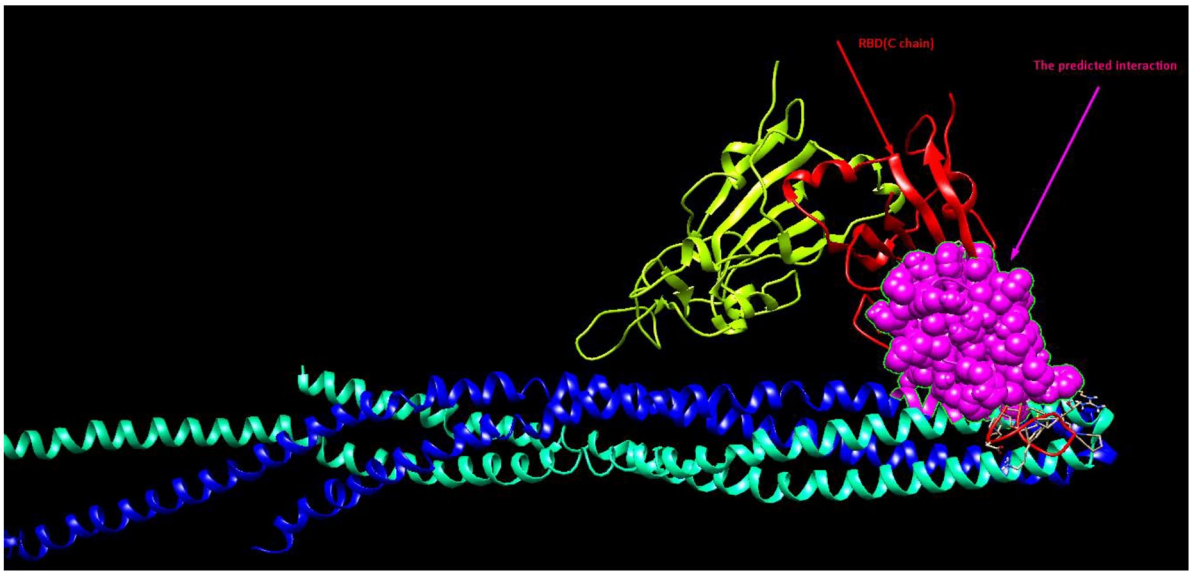

Figure 1. A screenshot for the interaction between bacteria protein PDB:1QU7 (both turquoise and navy blue) and SARS-CoV-2 receptor-binding domain (PDB:2GHV). The C-chain of spike receptor-binding domain interacts with bacteria protein. The places where possible interaction may occur are also indicated (in magenta color).

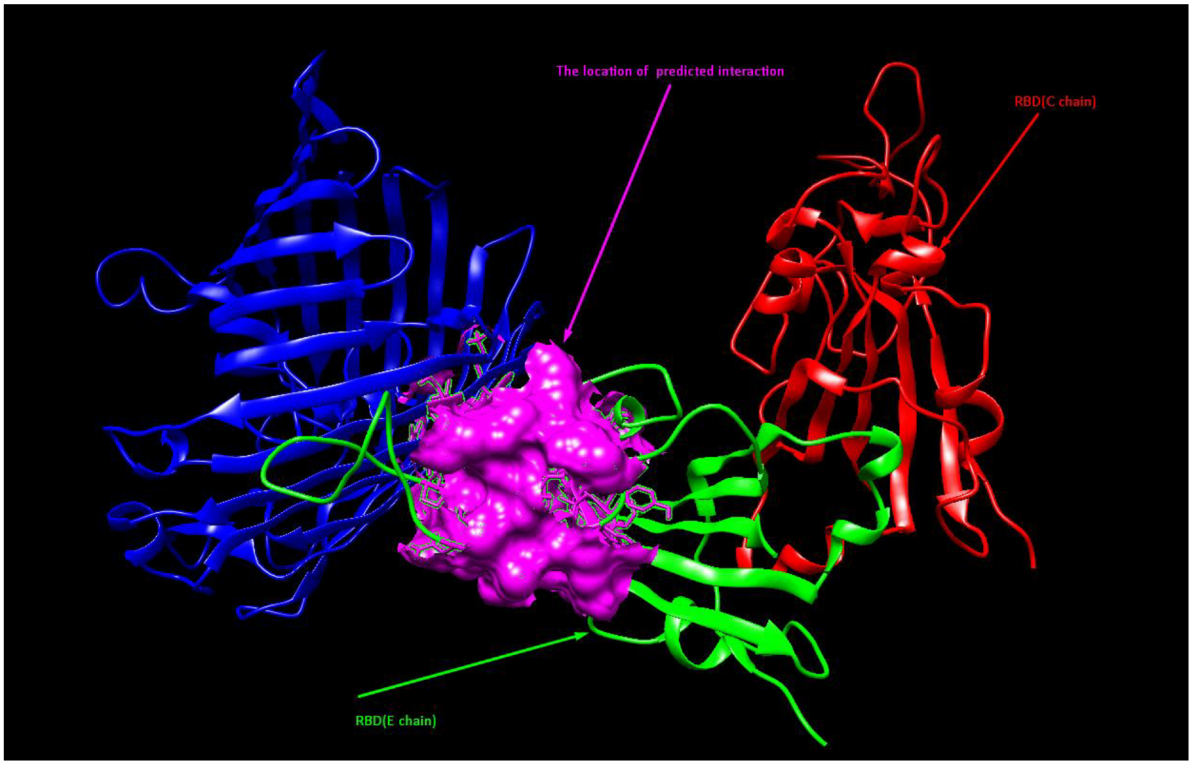

Figure 2. A screenshot for the interaction between bacteria protein PDB:2ZFG (Navy blue) and SARS-CoV-2 receptor-binding domain (PDB:2GHV). Only E- (It is shown in green color) chain of spike receptor-binding domain interacts with bacteria protein. The places where possible interaction may occur are also indicated (in magenta color).

transmission in our body. In addition, it is thought-provoking that SARS-CoV-2 went and connected to ACE2 since they deficit the key characteristics, such as cell structure, brain, and organelles. So, we assume that it is unlikely that this virus will come randomly and find ACE2.[6,8,42–44] This situation increases the likelihood that some partners, which bacteria may be one of these partners, as many studies support,[9,12,13,17] may occur when the SARS-CoV enters the human body. Severe acute respiratory syndrome coronavirus cannot reproduce in bacteria,[45] but this should not mean that SARS-CoV cannot bind to bacteria. There are very remarkable studies on the interaction between bacteria and viruses.[10,14-16,18,19] Uchiyama et al[15] found that

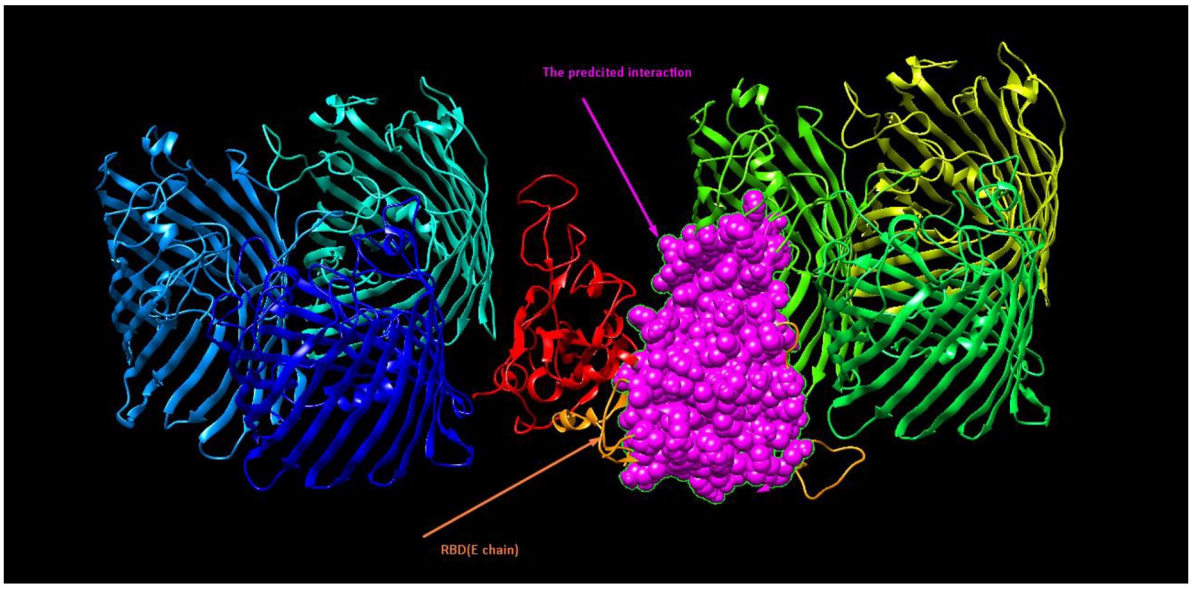

Figure 3. A screenshot for the interaction between bacteria protein PDB:2XG6 and SARS-CoV-2 receptor-binding domain (PDB:2GHV). Only E- (in orange color) chain of spike receptor-binding domain interacts with bacteria protein. The places where possible interaction may occur are also indicated (in magenta color).

Enteric bacteria help Rotavirus by enhancing both viral replication and virus binding/entry, and Pawlowski et al[16] revealed that *Mycobacterium tuberculosis* bacteria help the HIV by increasing HIV long-terminal repeat-driven transcription and HIV production during this binding/entry. Like direct interaction between bacteria and viruses, there are some examples of indirect interactions between bacteria and viruses. For instance, *S. pneumoniae* and *S. aureus* increase host cell adhesion molecules during the Rhinovirus effect.[17] During *S. pneumoniae* and Rhinovirus interaction, *S. pneumoniae* helps Adenovirus by increasing host cell adhesion molecules.[9,18] Petruk and her friends recently did a critical study and found that bacteria play a significant role in the COVID-19 outbreak. They found that SARS-CoV-2 spike protein bound to bacterial lipopolysaccharide and boosted proinflammatory activity.[46] The above interaction between bacteria protein and virus prompted us to investigate possible connections between the cytoplasmic domain of a serine chemotaxis receptor, OMPF porin, the teichoic wall acid, *E. coli* OmpF porin, Transport protein, Translocator EscV, and SARS-CoV-2 S protein from a structural perspective. Petruk and her friends found an interaction between bacteria lipopolysaccharide and SARS-CoV-2 spike protein. Our in silico results showed that more bacteria proteins might play a critical role in binding spike protein (Figures 1-3, Table 1). Our predicted proteins did not choose randomly; most of them are Gram-positive bacteria such as *Actinobacteria, Firmicutes*. They are located in a human body with a remarkable number. It is very important that Gram-positive bacteria have the wall teichoic acid that chelating agents and in some types to promote adhesion[47] because our in silico results show that the wall teichoic acid may bind spike protein (Table 1). This situation can be said as follows: there is a temporary bacteria-virus in interaction with this spike. By the function of this protein, the bacteria can carry the virus to ACE2. An important study found that, not surprisingly, mammalian cells are prone to bacterial attachment during a viral illness. Bacteria do attach using their surface proteins that bind to host extracellular matrix proteins, such as fibronectin, fibrinogen, vitronectin, and elastin, which often play a vital role in the initial adherence of bacteria to solid surfaces within the host.[24,25,48] In this viral epidemic, if the probability of mammalian cells to bind to bacteria increases, the interaction of bacteria and spike protein may increase. Moreover, the orientation of bacteria to cells can also increase spike-ACE2 interaction. Besides, it might be said that our results support this situation (Table 1, Figures 1–3). An example of bacterial attachment to mammalian cells is that *S. aureus* is especially significant for the plenty of microbial surface components recognizing adhesive matrix molecules that it can produce, including clumping factors A and B (ClfA/B), fibronectin-binding factors A and B (FnBA/B), and a collagen-binding protein (Cna). Also, *S. epidermidis* produces at least 2 adhesins that bind to fibronectin and the fibrinogen-binding protein. Flagella are implicated in adherence to *Vibrio cholerae, E. coli, P. aeruginosa*, and *Salmonella enterica*.[48-50] Moreover, according to our results (Table 1, Figures 1–3), we have found that both ACE2- and spike RBDs might bind to the bacterium proteins, such as serine chemotaxis receptor, OMPF porin, the wall teichoic acid polymerase TagF, OmpF porin in lipidic cubic phase, transport protein, and translocator protein. These may be thought of as when SARS-CoV-2 enters our body, bacteria may take a role in carrying this virus while heading toward cells by attaching microbial surface components recognizing adhesive matrix molecules. Angiotensin-converting enzyme 2 and spike RBD interaction docking score were predicted to be −267.98 by

HDOCK SERVER. As expected, the docking result between bacterial proteins (given in method with PDB code) and SARS-CoV's RBD was predicted better than ACE2 receptor-SARS-CoV-2's RBD (Table 1, Figures 1–3). That is, it is conceivable that the spike protein may direct and bind to ACE2 by binding to bacteria OMPs.

COVID-19 has come out as a multiorgan disease that causes damage to other organ systems, including the nervous and GI systems and respiratory disease.[51-53] Gastrointestinal system, which has a massive amount of bacteria with different species, is also important for the tendency and severity of COVID-19, and SARS-CoV-2 has been noted in the tissues of the entire GI tract. Severe acute respiratory syndrome coronavirus 2 infection, therefore, has a notable direct impact on the GI system, possibly as an important place for virus replication and activity.[54-56] It is not surprisingly known that ACE2 (the spike protein of SARS-CoV-2 binds to) is abundantly expressed in GI systems (the ileum and colon). Severe acute respiratory syndrome coronavirus 2 can readily infect the enterocytes.[56-58] This information situation makes us think that the gut is also an important target organ of SARS-CoV-2. Even the mortality rate of the COVID-19 is still vague; it is clear that it is far more deadly for adults aged 65 years and older than for children or younger people.[59-61] Like mortality age changing, ACE2 gene expression may vary with age.[62,63] The number of bacteria in humans can change depending on age, and remarkable changes in the gut microbiota occur early in life and during infection.[64-68] Besides, during COVID-19, the number of bacteria in GI systems was changed.[68] We think that the reason for this change may be an interaction between bacteria OMPs and spike protein. We found some predicted interaction between OMPs of bacteria and spike protein (Table 1, Figures 1–3). This interaction may damage bacteria's OMPs protein, and these OMPs may lose the characteristics they normally do. The predicted interaction between ACE2 and OMPs should not be ignored (Table 1) because the rate of infection may also be affected by the rate of ACE2 presence. There is already a bacterial protein and spike interaction in the literature.[46] Like the logic in this study, the more OMPs we found can interact with the spike (Table 1, Figures 1–3). If these interactions occur (Table 1, Figures 1–3), the probability that bacteria effectively transmit the virus may increase even more. Furthermore, according to the preliminary data of bacterial interaction with ACE2 (Table 1), the excess ACE2 may affect the infected rate. Bacteria can be affected differently in interaction SARS-CoV-2 because virus-promoting direct interactions occur when the virus exploits a bacterial component to facilitate penetration into the host cell may support our hypothesis.[54] For example, it is mentioned that the number of *Bacteroidetes, Firmicutes, Proteobacteria*, and *Actinobacteria* can change during COVID-19.[68] Like we said earlier, spike-bacteria interaction may damage bacteria OMPs protein, and OMPs may lose their characteristics what they normally do. As a result, other bacteria may replace the negatively affected bacteria. To give an example, although 9 species, including *Bacteroides stercoris, Bacteroides vulgatus, Bacteroides massiliensis, Bifidobacterium longum, Streptococcus thermophilus, Lachnospiraceae bacterium* 5163FAA, *Prevotella bivia, Erysipelotrichaceae bacterium* 6145, and *Erysipelotrichaceae bacterium* 2244. A were importantly enhanced, 6 species, namely, *Clostridium nexile, Streptococcus salivarius, Coprococcus catus, Eubacterium hallii, Enterobacter aerogenes*, and *Adlercreutzia equolifaciens*, were notably diminished in patients compared with those in the healthy controls.[68] In addition, bacteria are found in the mouth, skin, buccal mucosa, and nasal cavities outside the GI. Some of these bacteria are *Mutans streptococci, lactobacilli, S. pneumoniae, H. influenzae*, and *Moraxella catarrhalis*.[69-72] It was stated that expression of SARS-CoV-2 is very abundant in the nose.[73,74] An interaction between spike protein and bacteria in the nose and mouth may also be considered. Severe acute respiratory syndrome coronavirus 2 coming to body via the eyes, mouth, or nose may attach to the bacteria, and the bacteria can be affected by this situation and move toward the cells.[9,24,25] And again, as indicated in our results, it is possible that there is an interaction between the bacteria OMPs in the nose and mouth and ACE2, which is abundant in these 2 places[75] (Table 1, Figures 1–3). In this case, bacteria may play a mediating role in taking the virus to the relevant receptor. We think it is important to conduct more studies on these interactions to elucidate the subject further. We think it is important to conduct more studies on these interactions to elucidate the subject further.

It is quite important to prove the results obtained using bioinformatics tools in the wet laboratory and proving results in the wet laboratory will increase the reliability of bioinformatics results. The role of bacteria in this epidemic has been studied broadly and significant results have been obtained in the literature. The thought that bacteria could act in partnership with the SARS-CoV-2 virus was also predicted as a result of these situations.[75,76,77-79] The thought that bacteria could act in partnership with the SARS-CoV-2 virus was also predicted as a result of these situations. Petruk and her friends found an interaction between bacteria lipopolysaccharide and SARS-CoV-2 spike protein. Our in silico results showed that more bacteria proteins might play a critical role in binding spike protein.[46] Therefore, it will be very important to prove the results found in this study in the laboratory environment, and thanks to these results, this will have a different perspective on this epidemic caused by SARS-CoV-2. In addition, these wet laboratory results will not only determine the reliability of the results of our bioinformatics results but also the OMPs of some bacterial species will be viewed more skeptically in this outbreak. As a result, prejudices and disadvantages to the bioinformatics tools of our study will be eliminated.

Conclusion

We hypothesized that SARS-CoV-2 might have a relationship with bacteria as a result of bioinformatics analysis. Using

bioinformatics tools, we have obtained predicted results that the OMPs belonging to bacteria can interact with RBD of SARS-CoV-2 and ACE2. Therefore, the virus can use these bacteria as a carrier. The cooperation between these proteins and SARS-CoV-2 can help this virus transport or reproduce in our body. We think it is crucial to conduct more studies on these interactions to elucidate the subject further. Proving this relationship can provide a different perspective on how SARS-CoV-2 spreads in the host cell, and different paths can be followed in the diagnosis and treatment of the disease.

Author Contributions

Both authors reviewed the analysis and contributed to the preparation of the manuscript.

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