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Manipulated bio antimicrobial peptides from probiotic bacteria as proposed drugs for COVID-19 disease

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ABSTRACT

Coronavirus disease 19 (COVID-19) is the latest pandemic resulted from the coronavirus family. Due to the high prevalence of this disease, its high mortality rate, and the lack of effective treatment, the need for affordable and accessible drugs is one of the main challenges in this regard. It has been proved that RdRp, 3CL, Spike, and Nucleocapsid are the most important viral proteins playing vital roles in the processes of proliferation and infection. Therefore, we started studying a wide range of bio-peptides and then conducted molecular docking analyses to investigate their binding affinity for the inhibition of these proteins. After obtaining the best bio-peptides with the highest affinity scores, they were examined for further study and then manipulated to eliminate their side effects. Additionally, the molecular dynamic simulation was performed to validate the structure and interaction stability. The results of this study reveal that glycocin F from Lactococcus lactis and lactococcine G from Lactobacillus plantarum had the highest affinities to bind to the viral proteins, and the manipulation of their sequence also led to the side effects’ elimination. In addition, in some cases, their affinities to attach the SARS-CoV-2 proteins have increased. It seems that these two drugs which were discovered and designed, are optimal for treating the COVID-19 infection. However, experimental and pre-clinical studies are necessary to assay their therapeutic effects.

1. Introduction

The last outbreak of acute respiratory disease has been named COVID-19. Accordingly, it is the third documented disease spreading from other species infected by coronavirus to humans in the last two decades, which has led to a major epidemic [1]. The new virus originated from SARS-CoV with over 95% homology is called SARS-CoV-2 [2, 3]. A positive sense RNA from 60 nm to 140 nm in diameter and a Spike protein on its surface are the main features of this family of viruses. Human coronaviruses generally cause mild respiratory disease. Their genome consists of 6–11 open reading frames (ORFs), which can code non-structural and structural proteins. The proteins transcribed from these sequences are cut by 3-chymotrypsin-like (3CL), which is the main protease of SARS-CoV-2 [4]. Furthermore, M (membrane), E (envelope), N (nucleocapsid), and Spike proteins are its four main structural proteins [5].

Spike is responsible for binding to the host receptor and viral entry. SARS-CoV-2 infects host cells via binding to angiotensin-converting enzyme II (ACE 2) [6], which are dominantly present on the lung epithelial cells [7]. Thus, blocking the spike can be considered as an effective approach to prevent COVID-19 infection.

The N protein of SARS-CoV-2 plays many roles, including setting viral RNA synthesis through replication, forming helical ribonucleoproteins (RNPs) while packaging of the RNA genome, modulating the infected cell metabolism, and transcription. Therefore, this multifunctional RNA-binding protein is crucial for the transcription and replication of viral RNA [8]. The main functions of N protein are attaching to the viral RNA genome and collecting them into a large helical nucleocapsid structure or RNP complex [9]. Many studies have reported that N protein bound to leader RNA to maintain suitable RNA conformation for the replication and transcription of the viral genome [10]. Moreover, several investigations approved that N protein could regulate host-pathogen interactions, including apoptosis, host cell cycle progression, and actin reorganization [11–13]. The N protein is abundantly expressed during infection, so blocking it may modulate the infection [14–16].
3CL is a proven target for the inhibition of SARS-CoV and MERS-CoV. 3CL gene located at the 3’ end with excessive variability [17], is responsible for the replication of virus particles by cleaving the viral polyproteins at 11 distinct site to generate various non-structural proteins that are important for viral replication [18]. Therefore, as there are no host-cell proteases with the specificity to inhibit it, 3CL could be considered as another potential target for SARS-CoV-2 [19]. Moreover, SARS-CoV-2 expresses an RNA-dependent RNA polymerase (RdRp) to consider as another potential target for SARS-CoV-2 [19]. Moreover, therapeutic targets for this fatal disease [24].

In recent years, AMPs have been broadly used as a hopeful solution to conquer dangerous microorganisms. Many microorganisms produce these peptides as their innate immune response component to invade pathogens [25]. The use of AMP can be promising as a therapeutic tool for increasing viral infections, for which no authorized medication or treatment is available [26]. Notably, AMPs are used to treat viral-related infections such as zika virus (ZIKV), dengue virus (DENV) [27], and Lactoferrin is one of the AMPs playing an inhibitory role in the treatment of some viruses, including herpes simplex virus (HSV) [29], hepatitis C virus (HCV) [30], human immunodeficiency viruses (HIV) [31], rotavirus, and polio [32]. Based on the studies performed on the Lactoferrin antiviral activities and inhibitory effects of this AMP on SARS-CoV-2, this peptide has been recently introduced as a probable therapeutic target for COVID-19 [33].

Therefore, the use of AMPs, which have inhibitory effects on RdRp [21], 3CL [19], S [6], and N [14], as the known proteins of the SARS-CoV-2 virus, is a significant idea and a promising solution for the treatment of COVID-19. One study simulated a computational model of Spike and designed an HR2-based antiviral peptide to inhibit this protein. The affinity of this antiviral peptide was strong that could completely bind to HR1 of Spike to prevent the formation of the fusion core [34]. Another study has also found that α-helical peptides block ACE2, leading to the inhibition of SARS-CoV-2 [35]. In another in-silico study, the efficacies of lopinavir, ritonavir, hydroxychloroquine, and favipravir drugs were improved by adding TAT-peptide to them, and their interactions with 3CL have considerably increased [36].

Although previous studies were performed on AMPs and generally on the inhibition of one or two proteins of SARS-CoV-2, the present research was conducted to prevent four basic proteins of SARS-CoV-2 by bio-AMPs from probiotic sources as a new approach. As a result, it was revealed that glycocin F and lactococcine G are the best bio-AMPs. In order to prove the side effects of the common peptides with the best scores were checked for allergenicity, toxicity, anti-angiogenic, interleukin 4 inducing ability, anti-cancer ability, and hemolyciticy by AlgPred (https://webs.iiitd.edu.in/raghava/algpred/submission.html) [54], toxinpred (http://crdd.osdd.net/raghava/toxinpred/) [55], target antiangiog (http://codes.bio/targetantiong/) [56], IL-4pred (https://webs.iiitd.edu.in/raghava/i4pred/), ACPred (http://codes.bio/acpred/) [57], and hemopred (http://codes.bio/hemopred/) [58] databases, respectively. 2.2. Bio-AMPs extraction

In recent years, AMPs have been broadly used as a hopeful solution to conquer dangerous microorganisms. Many microorganisms produce these peptides as their innate immune response component to invade pathogens [25]. The use of AMP can be promising as a therapeutic tool for increasing viral infections, for which no authorized medication or treatment is available [26]. Notably, AMPs are used to treat viral-related infections such as zika virus (ZIKV), dengue virus (DENV) [27], and Lactoferrin is one of the AMPs playing an inhibitory role in the treatment of some viruses, including herpes simplex virus (HSV) [29], hepatitis C virus (HCV) [30], human immunodeficiency viruses (HIV) [31], rotavirus, and polio [32]. Based on the studies performed on the Lactoferrin antiviral activities and inhibitory effects of this AMP on SARS-CoV-2, this peptide has been recently introduced as a probable therapeutic target for COVID-19 [33].

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Although previous studies were performed on AMPs and generally on the inhibition of one or two proteins of SARS-CoV-2, the present research was conducted to prevent four basic proteins of SARS-CoV-2 by bio-AMPs from probiotic sources as a new approach. As a result, it was revealed that glycocin F and lactococcine G are the best bio-AMPs. In order to prove the side effects of the common peptides with the best scores were checked for allergenicity, toxicity, anti-angiogenic, interleukin 4 inducing ability, anti-cancer ability, and hemolyciticy by AlgPred (https://webs.iiitd.edu.in/raghava/algpred/submission.html) [54], toxinpred (http://crdd.osdd.net/raghava/toxinpred/) [55], target antiangiog (http://codes.bio/targetantiong/) [56], IL-4pred (https://webs.iiitd.edu.in/raghava/i4pred/), ACPred (http://codes.bio/acpred/) [57], and hemopred (http://codes.bio/hemopred/) [58] databases, respectively.
The best peptides according to their binding energy with SARS-COV-2.

Table 2

| Protein interactions | Docking score (kcal/mol) |
|----------------------|--------------------------|
| ACE2/SARS-COV-2 Spike | -128.8 ± 3.5 |

Table 3

| Peptide code | Peptide name | Microorganism | Sequence | Dock score (kcal/mol) |
|--------------|--------------|---------------|----------|-----------------------|
| 2MV1         | Bacteriocin plantaricin ASMI subunit beta | Lactococcus lactis | KPAWCOYTLMACGAGYDSGTCYMYSHCFGVKSSGGSSYYHC | -149.7 ± 3.2 |
| 2JKP         | Bacteriocin lactococcine G | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 2LJ7         | Defensin Le-def | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 2LJ5         | Gallicacin-2 | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 2KUY         | Bacteriocin glycocin F | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 1H5O         | Crotamine | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 2RU0         | Actinomycin | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |
| 1Z64         | Pleurocidin | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -143.7 ± 3.5 |

Table 4

| Peptide code | Peptide name | Microorganism | Sequence | Dock score (kcal/mol) |
|--------------|--------------|---------------|----------|-----------------------|
| 2JPK         | Bacteriocin lactococcine G subunit beta | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -151.4 ± 9.8 |
| 2MLU         | LspB | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -151.4 ± 9.8 |
| 5ESQ         | Snakin-1 | Solanum tuberosum | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -151.4 ± 9.8 |

Table 5

| Peptide code | Peptide name | Microorganism | Sequence | Dock score (kcal/mol) |
|--------------|--------------|---------------|----------|-----------------------|
| 2KUY         | Bacteriocin glycocin F | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2KET         | Cathelicidin-6 | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 1PXQ         | Subtilisin-A | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2KEG         | Bacteriocin PlnK | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2JOX         | Moronecidin | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2JPK         | Bacteriocin lactococcine G subunit beta | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 4GV5         | Crotamine Ile-19 | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 1CW6         | Bacteriocin leucocin-A | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2G9P         | M-zotadixin-Li2a | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 1RKK         | Polyphemusin-1 | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 1EWS         | Corticostatin-related peptide RK-1 | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |

Table 6

| Peptide code | Peptide name | Microorganism | Sequence | Dock score (kcal/mol) |
|--------------|--------------|---------------|----------|-----------------------|
| 2KUY         | Bacteriocin glycocin F | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 1BRZ         | Defensin-like protein | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2MV1         | Bacteriocin plantaricin ASMI subunit beta | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |
| 2JKP         | Bacteriocin lactococcine G subunit beta | Lactococcus lactis subsp. lactis | KKWGWLAWDVPAYEIKGFKGAIKEGNKDKWKNI | -130.9 ± 2.5 |

3. Results

The results of docking analyses are presented in Tables 2-6. The affinity between ACE2 and SARS-CoV-2 Spike protein has been determined as a criterion for comparison. Notably, the Bacteriocin plantaricin ASMI peptide had the best affinity to the SARS-CoV-2 Spike. Moreover, Bacteriocin lactococcine G had the best affinity for RdRp protein, and Bacteriocin glycocin F had the highest binding affinity for 3CL and N proteins gained from StraPep and PhyAMP databases.

To investigate the inhibitory effect of bio-AMPs on the Spike, the
binding energy of the Spike to the ACE2 was used as a control sample, which was \(-128.8 \pm 3.5\) kcal/mol (Table 2). In addition, the binding energy of the Bacteriocin plantaricin ASM1 peptide and the Spike was \(-149.7 \pm 3.2\) kcal/mol. All the peptides shown in Table 3 have a higher affinity for Spike compared to ACE2, and as mentioned earlier, Bacteriocin plantaricin ASM1 peptide had the highest affinity for Spike compared to the other peptides.

The ACE2 receptor was used as a control to compare the affinity of peptides with Spike, but unfortunately, because the ligands that normally bind to 3CL, RdRp, and N protein of SARS-CoV-2 are chemical
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Table 7

| Peptide name | Sequence | Spike Docking Score (kcal/mol) | RdRp Docking Score (kcal/mol) | 3CL Docking Score (kcal/mol) | N Docking Score (kcal/mol) | Allergenisity | Anticancer | Antiangiogenic | Hemolytic |
|--------------|----------|-------------------------------|-------------------------------|-------------------------------|---------------------------|---------------|-------------|---------------|-----------|
| 2JPK - Wild  | KKWKGLLAWVDPAYEFIKGFGKGAIKEGNKDKWKNI | -130.4 ± 9.8 | -108.9 ± 7.5 | -98.0 ± 11.2 | -128.3 ± 13.2 | ACP | Non | Non | Non |
| 2JPK - Mutant | RKWPWLAWVEGAYEYIKGWGKGAVREGQKEKWRNV | -149.2 ± 3.7 | -126.5 ± 2.5 | -132.6 ± 13.2 | -128.3 ± 13.2 | ACP | Non | Non | Non |
| 2KUY - Wild | KPAWCWYTLAMCGAGYDSGTCDYMYSHCFGIKHHSSGSSSYHC | -133.6 ± 8.6 | -100.8 ± 10.2 | -133.6 ± 8.6 | -128.3 ± 13.2 | ACP | Non | Non | Non |
| 2KUY - Mutant | RWPGWYWIALGEGCGGTVGTVGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIGTVTIM
4. Discussion

Since the outbreak of COVID-19, humans have found that few options still exist for the treatment of life-threatening coronavirus infections. The outbreaks of SARS and MERS-CoV have led to performing more studies on the coronaviridae family; however, there is no definite drugs to treat these coronaviruses yet. Over the last 17 years, coronaviruses have shown a transient nature and led to epidemics; this feature prevents prototype coronavirus inhibitors from their development to the preclinical stage. Therefore, it seems that finding broad-spectrum inhibitors to decrease the effects of human coronavirus infection is a challenging research focus.

SARS-CoV-2 enters host cells by binding to ACE2 through the Spike, then viral RNA is transcribed by RdRp, viral RNA is replicated and transcribed, and the N protein is finally synthesized in the cytoplasm. Whereas other viral SPs such as the Spike, M, and E proteins are transcribed and translated in the endoplasmic reticulum (ER). The important proteins of SARS-CoV-2 are processed by 3CL protein, and they are then

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Fig. 3. Spike and 2JPK interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin lactococcin-G subunit beta-peptide with Spike protein of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2JPK/Spike complex; peptide chain is colored in pink, Spike is presented in plum, and the important amino acids of Spike protein in the interaction are exhibited in rosy brown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Spike and 2KUY interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin glycocin F peptide with Spike protein of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2KUY/Spike complex; peptide chain is colored in cyan, Spike is presented in plum, and the important amino acids of Spike protein in the interaction are exhibited in rosy brown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
assembled at the ER–Golgi intermediate compartment (ERGIC) to form a mature virion. Finally, the nascent virion is released from the host cells [59]. Therefore, the inhibition of each of these proteins could be known as a suitable therapeutic target for combating and controlling COVID-19.

In an investigation, nearly 32,297 potential anti-viral phytochemicals were screened to select the top nine hits that can prevent 3CL activity and replication. Accordingly, many of these medicinal plant compounds have been already used to treat various viral diseases successfully [47]. Another in silico study has also presented that ligand-binding is strikingly similar in SARS-CoV and SARS-CoV2 main proteases, and also confirmed that a derivation of chlorophenyl-pyridyl-carboxamide has the strongest affinity to 3CL [48]. Moreover, an investigation identified top compounds among the natural products by virtual screening, which showed that simeprevir and loniflavone had the highest affinities to Spike, and conivaptan and amyrin had the highest ones to the nucleocapsid protein [52].

Fig. 5. 3CL and 2JPK interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin lactococcin-G subunit beta-peptide with 3CL of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2JPK/3CL complex; peptide chain is colored in pink, 3CL is presented in khaki, and the important amino acids of 3CL protein in the interaction are exhibited in light green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 6. 3CL and 2KUY interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin glycocin F peptide with 3CL of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2KUY/3CL complex; peptide chain is colored in pink, 3CL is presented in khaki, and the important amino acids of 3CL protein in the interaction are exhibited in light green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
studies by investigating mechanistic studies to clinical trials for COVID-19 presented that remdesivir is a potential drug as a SARS-CoV-2 RNA-chain terminator, which can effectively stop its RdRp [60]. Finally, the Food and Drug Administration (FDA) approved remdesivir as an effective drug on the inhibition of SARS-CoV-2 reproduction [61].

In previous studies, inhibitory agents were usually used for one or two proteins, while in this study, bio-AMPs that had probiotic properties were used to inhibit the viral underlying proteins, including RdRp, 3CL, Spike, and N proteins.

The physicochemical and structural properties of bio-AMPs are essential factors in determining their specificity towards the destination cells. Correspondingly, they are efficient agents with different structural and antimicrobial features that can serve as one of the most hopeful future drug candidates to treat COVID-19 infections. For example, Oleg

![Fig. 7. N protein and 2JPK interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin lactococcin-G subunit beta-peptide with N protein of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2JPK/N protein complex; peptide chain is colored in pink, N protein is presented in light gray, and the important amino acids of N protein in the interaction are exhibited in purple. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)](image1)

![Fig. 8. N protein and 2KUY interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin glycocin F peptide with N protein of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2KUY/N protein complex; peptide chain is colored in cyan, N protein is presented in light gray, and the important amino acids of N protein in the interaction are exhibited in purple. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)](image2)
Kit and Yuriy Kit gathered some information on a group of natural peptides with various origins. They proposed that some peptides such as Angiotensins that regulate blood pressure, Bradykinin as an inflammatory mediator, and Beta-casokinin 1 as a bioactive component of milk and dairy products, could be suggested as novel drugs in the treatment of COVID-19 disease \cite{40,41}. Perhaps the best option for choosing the source of peptides is using probiotic bacteria, because they are naturally beneficial to human health, so they can also be used in people’s daily diet, which have been stated as a food complement for human health through making useful compounds \cite{62}.

In this study, after the investigation of many bio-AMPs, and surveying their effects on Spike, RdRp, 3CL, and N proteins, two common bio-peptides, named glycocin F and lactococcine G, from \textit{Lactococcus lactis} and \textit{Lactobacillus Plantarum} were selected with the highest affinity to these proteins, respectively. The other products from these two probiotics with no side effects are used in the food industry.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{RdRp and 2JPK interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin lactococcin-G subunit beta-peptide with RdRp of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2JPK/RdRp complex; peptide chain is colored in pink, RdRp is presented in light blue, and the important amino acids of RdRp in the interaction are exhibited in light gray. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{RdRp and 2KUY interaction. A: 2-D picture of hydrophobic interactions and H-bonds of Bacteriocin glycocin F peptide with RdRp of SARS-CoV-2 virus using LigPlot. B: 3-D structure of 2KUY/RdRp complex; peptide chain is colored in pink, RdRp is presented in light blue, and the important amino acids of RdRp in the interaction are exhibited in light gray. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)}
\end{figure}
As claimed by the previous studies, taking vitamin D prevents the proliferation of the SARS-CoV-2 virus, and to the best of our knowledge, dairy products, in turn, contain vitamin D [63]. Notably, using dairy products comprising the *Lactococcus lactis* and *Lactobacillus Plantarum* that produce glycocin F and lactococcine G, may consequently double the inhibitory effect with the consumption of vitamin D.

According to the advantages of the two obtained bio-AMPs, we can produce dairy products based on *Lactococcus lactis* and *Lactobacillus Plantarum* probiotic bacteria to control and prevent COVID-19 disease. Furthermore, the mentioned bio-AMPs could be used as a synthetic medicine with different dosages. If bio-AMPs need to be used as a drug with high concentration, their side effects need to be checked. Thus, glycocin F and lactococcine G were checked in terms of their probable negative effects, and the result reveals that the allergenicity is the most important side effect. Therefore, to solve this problem, peptide manipulation was performed to achieve the proper condition.

Due to the cost of the COVID-19 pandemic for countries, developing a treatment with low cost is very precious. *Lactococcus lactis* and *Lactobacillus Plantarum* probiotic bacteria’s products, which are abundantly found in dairy products, can be consumed to control this fatal disease. Also, the manipulated glycocin F and lactococcine G can be used as therapeutic targets for the inhibition of SARS-CoV-2 virus entrance, replication, and development in a variety of possible drug delivery

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**Fig. 11.** The RMSD graph for the entire MD simulation timescale (45 ns) is illustrated for both 2JPK peptide and Spike protein of SARS-CoV-2 complex.

**Fig. 12.** The RMS fluctuation graph. A: the fluctuation of Spike protein amino acids. B: the fluctuation of amino acids of 2JPK peptide.

**Fig. 13.** 3-D interaction of 2JPK/Spike protein interaction after the mutation and MD simulation obtained from Discovery Studio. The important amino acids of Spike protein are colored in black and shown in ball and stick styles. The important amino acids of the peptide involved in the interaction are also displayed in three colors as pink, green, and purple. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
mechanisms.

5. Conclusion

Based on this conducted study, it was shown that glycinin F from *Lactococcus lactis* and lactococcin G from *Lactococcus Plantarum* are the common peptides with high-affinity binding to SARS-CoV-2 S, N proteins, and 3CL protease. Moreover, lactococcin G was found to have a high affinity to RdRp protein. The present study revealed that the optimization of glycinin F and lactococcin G can convert these two bio-peptides to suitable therapeutics factors for SARS-CoV-2 protein inhibition with no side effects. Thus, these peptides could be considered as potential drugs to control COVID-19 disease. However, more experimental and pre-clinical studies on peptides’ purification, characterization, and mutation, as well as on the possibility of their anti-viral effects on the SARS-CoV-2 virus are needed to use them as novel medicines for COVID-19.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviation

Coronavirus disease 19 (COVID-19) 3-chymotrypsin-like (3CL) M (membrane) E (envelope) N (nucleocapsid) open reading frames (ORFs) angiotensin-converting enzyme II (ACE 2) ribonucleoproteins (RNP}s) RNA-dependent RNA polymerase (RdRp) bio-antimicrobial peptides (bio-AMPs) zika virus (ZIKV) dengue virus (DENV) (27) Influenza A virus (IAV) herpes simplex virus (HSV) (29) hepatitis C virus (HCV) (30) human immunodeficiency viruses (HIV) Root-Mean-Square Deviation (RMSD) and Root-Mean-Square Fluctuation (RMSF) Food and Drug Administration (FDA)

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