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# Exploring the binding capacity of lactic acid bacteria derived bacteriocins against RBD of SARS-CoV-2 Omicron variant by molecular simulations

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**ABSTRACT**

The changes in the SARS-CoV-2 genome have resulted in the emergence of new variants. Some of the variants have been classified as variants of concern (VOC). These strains have higher transmission rate and improved fitness. One of the prevalent were the Omicron variant. Unlike previous VOCs, the Omicron possesses fifteen mutations on the spike protein's receptor binding domain (RBD). The modifications of spike protein's key amino acid residues facilitate the virus' binding capability against ACE2, resulting in an increase in the infectiousness of Omicron variant. Consequently, investigating the prevention and treatment of the Omicron variant is crucial. In the present study, we aim to explore the binding capacity of twenty-two bacteriocins derived from Lactic Acid Bacteria (LAB) against the Omicron variant by using protein-peptidedocking and molecular dynamics (MD) simulations. The Omicron variant RBD was prepared by introducing fifteen mutations using PyMol. The protein-peptide complexes were obtained using HADDOCK v2.4 docking webserver. Top scoring complexes obtained from HADDOCK webserver were retrieved and submitted to the PRODIGY server for the prediction of binding energies. RBD-bacteriocin complexes were subjected to MD simulations. We discovered promising peptide-based therapeutic candidates for the inhibition of Omicron variant for example Salivaricin B, Pediocin PA 1, Plantaricin W, Lactococcin mmfii and Enterocin A. The lead bacteriocins, except Enterocin A, are biosynthesized by food-grade lactic acid bacteria. Our study puts forth a preliminary information regarding potential utilization of food-grade LAB-derived bacteriocins, particularly Salivaricin B and Pediocin PA 1, for Covid-19 treatment and prophylaxis.

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SARS-CoV-2; omicron variant; lactic acid bacteria; bacteriocins; protein-protein docking; Pediocin PA 1; Salivaricin B

## Introduction

COVID-19 still threatens human lives and remarkably changed public health, with over 407 million cases and 5.8 million deaths worldwide as of February 11, 2022 (COVID Live—Coronavirus Statistics—Worldometer, n.d.). The emergence of new variants undermines the effectiveness of the vaccine. SARS-CoV-2 infection occurs when the spike (S) protein of the virus binds to human angiotensin-converting enzyme II (ACE2) proteins. The spike protein is evolutionarily the most mutated virus component. Evolutionary pressure has dominated both to increase (or not decrease) the binding affinity for ACE2 and to evade neutralization of antibodies. Some of the popular VOCs have been the Alpha, Beta, Gamma, Delta, and Omicron. In particular, it has been determined that the Omicron variant, which has recently taken over the world, is immune to most therapeutic monoclonal and large-extended vaccine-derived antibodies (Planas et al., 2022). Vaccine-induced immunity and protection appear to be waning over time (Dolgin, 2021). In order to deal with the ongoing COVID-19 pandemic; new, safe, low-cost, and quickly implementable techniques are still required (Wischmeyer et al., 2022).

The Omicron variant (B.1.1.529.1 or BA.1) was first identified in Botswana in November 2021 (Viana et al., 2022) which prompted significant concern (Iketani et al., 2022). The Omicron was shown to transmit 100 fold more compared to delta variant and evades preexisting immunity (Fan et al., 2022)(Rao & Singh, 2021). Due to its altered cell tropism, the Omicron variant has been observed to be less harmful, but it is more resistant to vaccinations, convalescent serum, and the majority of antibodies (Fan et al., 2022). The SARS-CoV-2 Omicron variant contains fifty-one mutations, thirty-three of which are positioned on the S protein. The Omicron variant possesses thirty-seven point modifications in the S protein, fifteen of them are located in RBD (Cameroni et al., 2022). It is believed that modifications to certain amino acid residues on the S protein's RBD can enhance the virus' binding to

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ACE2, resulting in a significant increase in the infectiousness of the Omicron variant (Karim & Karim, 2021). .

Gastrointestinal (GI) diseases such as nausea, diarrhea, vomiting, and respiratory diseases like fever, cough, and wheezing usually happen together in COVID-19 (Guan et al., 2020; Huang et al., 2020; Wang et al., 2020; M. Zhang et al., 2021). In addition, SARS-CoV-2 is being isolated from both in oral swabs ((Teo et al., 2021)), as well as rectal swabs and stool samples of infected patients (Holshue et al., 2020; Tang et al., 2020; Kaijin et al., 2020). This perhaps could reveal a bidirectional communication between gut and lungs (i.e. also known as gut-lung axis) in COVID-19 (Ahlawat et al., 2020; Allali et al., 2021; Dhar & Mohanty, 2020; He et al., 2020). The incidence of GI symptoms in COVID-19 patients could be caused by injured tissues or organs due to immune response (Lehtoranta et al., 2014). As an alternative, ACE2 is the major host cell receptor of SARS-CoV-2 (Ciaglia et al., 2020). In addition to the lung, the intestine is the other GI organ in which ACE2 is expressed. Therefore, colonization of ACE2 receptors in the gut via the virus potentially causes SARS-CoV-2 associated GI tract symptoms (Olaimat et al., 2020). The clinical and laboratory research indicates that probiotics can prevent or reduce the severity of respiratory infections, and that gut microbiota influence COVID-19 transmission risk and symptoms. Thus, probiotics are a viable strategy for preventing and reducing the risk of COVID-19 (Walton et al., 2021).

The positive impact of probiotics against ACE is well described (Robles-Vera et al., 2017). During fermentation, probiotics biosynthesize bio-peptides also called bacteriocins with ability to inhibit the ACE via blocking the active sites of enzyme (M. Ayyash et al., 2020; M. M. Ayyash et al., 2012). In addition, cell debris of non-alive probiotics also functions as ACE inhibitors (Miremadi et al., 2014). These findings perhaps indicate that probiotics might serve as a blocker to the ACE receptor, an entry for SARS-CoV-2 for attacking cells of GI (Olaimat et al., 2020). The hypothesis of utilizing drugs for blocking the ACE receptors for COVID-19 treatment was suggested by Fernández-Fernández (Fernández-Fernández, 2020). Similarly, Imai et al. proposed that use of ACE blocking agents reduces respiratory distress syndrome (Imai et al., 2008).

Probiotics are live microbes. They provide beneficial effects to host health when ingested in sufficient numbers of $10^8$-$10^{10}$ cfu/day (FAO/WHO, 2002). Probiotics were being suggested as antimicrobial agents against several pathogenic bacteria, though indirect or direct antiviral performance was reported for certain probiotic strains (Al Kassaa, 2017). Bacteriocins are peptidic molecules synthesized by several probiotic bacteria to kill other bacteria. These peptide-based compounds play an important role in the regularization of human microbiota and the defense of the GI tract (Fliss et al., 2011). Although bacteriocins' antibacterial activity has partially been described, their antiviral activity remains to be better understood (Al Kassaa, 2017).

In the literature, there are a hand full of papers evaluating lactic acid bacteria derived bacteriocins against SARS-CoV-2 and those papers are mostly targeting the wild type strain apart from our recent report (Erol et al., 2021) which put forth a comparative effect of bacteriocins against, Alpha, Beta and Delta mutants (Erol et al., 2021). It was reported that bacteriocins of salivaricin P, salivaricin B, pediocin PA-1 showed significant binding activities against the RBD of SARS-CoV-2 spike protein across alpha, beta and delta variants with highest predicted binding affinities were achieved against the Delta variant (Erol et al., 2021). However, we did not come across any scientific report estimating the binding affinity of bacteriocins against the RBD of Omicron variant which is the predominant mutant strain known to be highly infectious causing order of magnitude COVID-19 cases across the globe. Thus, the present study explores the binding capacity of twenty-two LAB derived bacteriocins against the RBD of SARS-CoV-2 Omicron variant by using protein-peptide docking and MD simulations.

## Materials and methods

All 22 bacteriocins were taken from our latest study (Erol et al., 2021). The details about the preparation protocols of the bacteriocins were given in the following publication (Erol et al., 2021). Following 15 mutations were introduced; Y505H, N501Y, Q498R, G496S, Q493K, E484A, T478K, S447N, G446S, N440K, K417N, S375F, S373P, S371L, and G339D using PyMol to obtain Omicron variant. HADDOCK v2.4 was used to predict binding modes of the bacteriocins in protein–protein docking simulations (Zundert et al., 2016). Same active residues that we defined in our previous paper (Erol et al., 2021) were used (i.e., the active residues on RBD: TYR505, GLY502, ASN501, THR500, GLN498, GLY496, GLN493, PHE490, TYR489, ASN487, PHE486, GLU484, GLY476, ALA475, PHE456, LEU455, TYR453, TYR449, GLY446, and LYS417 at the RBD, for the active residues for bacteriocins, all residues set as active). Passive residues (i.e., the residues that unwanted in the interaction interface) were automatically defined around the active residues. The parameter sets for protein-peptide docking was default settings and model refinement were used on the HADDOCK server. Top complexes from HADDOCK (i.e., based on calculated lowest HADDOCK scores) were retrieved. For the calculation of binding energies, the PROtein binDIng enerGY prediction (PRODIGY) server was used (Xue et al., 2016). According to PRODIGY $\Delta G$ energies, five top-scored bacteriocins were selected, 139586573 (Plantaricin W), 139587481 (Enterocin A), 139588229 (Lactococcin Mmfii), 16198259 (Salivaricin B), and 56842033 (Pediocin PA-1). In the docking calculations, we used RBD without glycan at N343 position. After top-docking scored poses are obtained, we introduced beta-D-mannopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose at ASN343 position by copying the glycan from 7DMU crystal structure (Higuchi et al., 2021). Glycosylated RBD-bacteriocin complexes were simulated for 500 ns. MD simulations for each system were repeated eight times ($5 \times 0.5$ μs x $8 = 20$ μs for bacteriocin-bounded systems, RBD-only systems were repeated for two times ($2 \times 0.5$ μs $= 1$ μs), in total 21 μs for all systems). For the solvation of RBD-Bacteriocin complexes, TIP3P water model was used (Jorgensen et al., 1983) in an orthorhombic box. Physiological salt concentration (0.15 M NaCl) was used to neutralize the simulation box. MD

simulations were run under NPT ensemble with Martyna Tobias Klein (MTK) barostat (Martyna et al., 1994) and Nosé–Hoover thermostat (Hoover, 1985; Nosé, 1984) using Desmond as MD engine (D. E. Shaw Research, New York, NY). 5000 frames were recorded for each simulation repeats. A total of $8 \times 5000 = 40000$ frames were collected for each system. In RBD-only systems $2 \times 5000 = 10000$ frames collected. Molecular Mechanics Generalized Born Surface Area (MM/GBSA) method was used with 200 frames out of 5000 frames for free energy calculations. In relative binding free energy calculations VSGB 2.0 implicit model was utilized (Li et al., 2011). MM/GBSA calculations were run using Prime module which is available under the Schrodinger's Maestro package (Schrodinger LLC) (Jacobson et al., 2002, 2004). The highly populated states were identified by clustering frames into clusters with agglomerative hierarchical method in TTClust code (Tubiana et al., 2018). Cluster analyses were performed on concatenated trajectories (40000 frames for each). Backbone atoms were used in alignment, and Ward algorithm was utilized to cluster frames into 15 members. The most populated structures were selected and visualized using PyMol.

## Results

### The tightest binding pose observed in the Salivaricin B (16198259) system

The tightest binding mode was observed in Salivaricin B. The binding free energy from protein-protein docking simulations was predicted as −11.8 kcal/mol. Second lowest predicted binding free energy was observed as −11.6 kcal/mol in Pediocin PA-1. Plantaricin W binding to Omicron-RBD was calculated as −10.4 kcal/mol (Table 1). However, when we divide the predicted binding energy to heavy atoms (known as Ligand Efficiency score), Lactococcin mmfii and Plantaricin D were the first best scoring bacteriocins (-0.14 kcal/mol). Enterocin A was the third best one in terms of ligand efficiency scores, where its binding to Omicron-RBD was predicted as −0.13 kcal/mol. Figure S1 shows the top docking poses achieved for all studied systems.

### The lowest RBD backbone atom RMSD observed in the Enterocin A (139587481) system

The lowest average backbone RMSD for the RBD observed in Enterocin A system as 2.10 ± 0.22 Å (the repeat averages were 2.20, 2.01, 1.94, 2.49, 1.92, 1.84, 2.09, and 2.31). Second lowest average backbone

RMSD was calculated as 2.11 ± 0.05 Å in RBD-only system (2.07, and 2.15). The third lowest RMSD was calculated as 2.22 ± 0.41 Å in Salivaricin B (2.04, 3.16, 1.94, 2.17, 1.94, 1.98, 2.42, and 2.12). The RMSD of backbone atoms of the RBD in the Plantaricin W system calculated as 2.24 ± 0.24 Å (2.18, 2.09, 2.22, 2.38, 2.48, 2.52, 1.78, and 2.29). The averages for the Lactococcin Mmfii, and Pediocin PA-1 found as 2.40 ± 0.25 Å (2.51, 2.29, 2.11, 2.96, 2.27, 2.33, 2.39, and 2.35), and 2.44 ± 0.41 Å (2.80, 2.12, 2.86, 3.00, 1.90, 2.42, 2.31, and 2.11), respectively. Figure 1 shows the RBD's backbone RMSD distribution of all simulated Omicron RBD systems.

Table 1. Omicron RBD–bacteriocin docking results (kcal/mol).

| Pubchem ID | Prodigy – dG | Bacteriocin name |
|---|---|---|
| 16198259 | −11.8 | Salivaricin B |
| 56842033 | −11.6 | Pediocin PA 1 |
| 139586573 | −10.4 | Plantaricin W |
| 139588229 | −10.3 | Lactococcin mmfii |
| 139587481 | −10.3 | Enterocin A |
| 139587600 | −9.7 | Salivaricin P |
| 139587056 | −8.9 | Leucocin B-TA11A |
| 139584476 | −8.9 | Leucocin B-TA33a |
| 139586697 | −8.8 | Plantaricin D |
| 139588080 | −8.8 | bavaricin A |
| 132535900 | −8.6 | Plantaricin JLA-9 |
| 139588082 | −8.5 | Mutacin |
| 139586357 | −8.5 | Enterocin CRL 35 |
| 139586277 | −8.4 | Gassericin B3 |
| 139583933 | −8.3 | Carnobacteriocin B2 |
| 146684209 | −8.3 | Plantaricin GZ1-27 |
| 139586327 | −8.3 | Gassericin B1 |
| 139583454 | −8.3 | Leucocin C-TA33a |
| 101561458 | −8.2 | Caseicin A |
| 139586139 | −8.2 | Enterocin EJ97 |
| 16730443 | −8.1 | Caseicin B |
| 139586609 | −7.8 | Gassericin B2 |

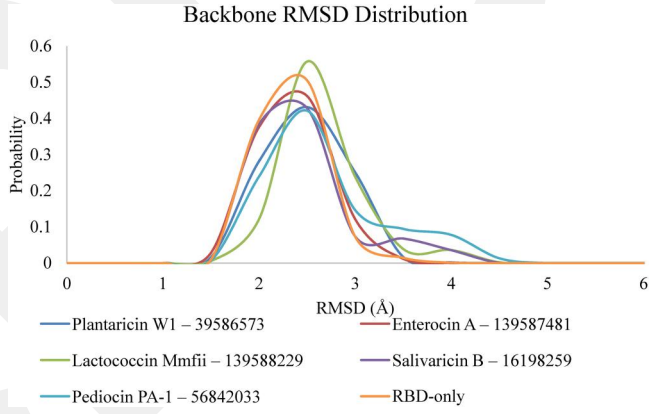


Figure 1. Backbone RMSD distribution of the RBD in all systems.

In the all studied RBD-bacteriocin systems, the distribution of backbone RMSD values was observed to be similar.

### The lowest bacteriocin backbone atom RMSD observed in the Plantaricin W (139586573) system

The lowest average backbone RMSD for the bacteriocin observed in Plantaricin W system as 2.90 ± 0.72 Å (2.04, 2.58, 3.92, 3.22, 2.37, 3.78, 2.16, and 3.15). The second lowest RMSD calculated in Salivaricin B system as 3.38 ± 1.05 Å (2.50, 4.67, 3.01, 2.06, 4.87, 3.92, 3.56, and 2.48). The system that has the third lowest average RMSD was Lactococcin Mmfii, where 3.59 ± 1.36 Å RMSD value obtained (5.51, 3.33, 1.36, 2.55, 3.04, 4.30, 5.12, and 3.49). The backbone RMSD averages for the Enterocin A and Pediocin PA-1 found as 3.94 ± 0.69 Å (3.05, 3.49, 4.48, 3.14, 3.73, 4.78, 4.09, and 4.72) and 5.65 ± 0.99 Å (5.04, 7.60, 5.18, 5.65, 4.75, 6.65, 4.86, and 5.51). Figure 2 shows the backbone RMSD distribution of the bacteriocins.

When the backbone RMSD distributions of bacteriocins were examined, it was observed that Pediocin PA-1 was

clearly separated from the others in the negative direction (with a higher RMSD average). Although Lactococcin Mmfii has two shoulders at high RMSD values, it was placed on the far left in the scatterplot.

### The lowest MM/GBSA binding energy observed in the Pediocin PA-1 (56842033) system

MD trajectories were used in MM/GBSA calculations. From each simulation, we extracted 200 frames and in total 1600 ($8 \times 200$) frames were calculated. The lowest average was observed in Pediocin PA-1 system as $-111.52 \pm 17.43$ kcal/mol. All averages and standard deviations were given in Table 2. Figure 3 shows the MM/GBSA scores as a box and whisker chart. MM/GBSA energies were plotted for each run and provided in Figures S2–S6 in Supplementary Material, respectively for all studied systems.

### The highest hydrogen bonds number observed in the Pediocin PA-1 (56842033) system

Concatenated trajectories were used in hydrogen bond analyses. The number of hydrogen bonds observed in RBD - Pediocin PA-1 system was the highest compared to other simulated complexes. $7.05 \pm 3.42$ hydrogen bonds observed in this system. The lowest number of hydrogen bond was observed in Enterocin A system, $3.03 \pm 1.92$ H-bonds observed. The number of hydrogen bonds $4.24 \pm 2.11$, $4.56 \pm 2.38$, and $4.90 \pm 2.42$ were observed in Lactococcin Mmfii, Plantaricin W, and Salivaricin B, respectively. (Figure 4)

### Clustering analyses reveal most populated binding modes

After trajectory clustering, representatives for each system were obtained. The selected representatives were observed in 15.13%, 23.00%, 15.85%, 12.97%, and 12.39% for the Plantaricin W, Enterocin A, Lactococcin Mmfii, Salivaricin B, and Pediocin PA-1. (Figure 5)

All polar interactions were evaluated in all RBD-Bacteriocin systems. In 139586573, ARG6 of Plantaricin W formed two interactions with E471 of RBD. Side chain of ASN9 interacted with the N481 backbone of RBD. ASN13 of Plantaricin W formed two interactions with the backbone atoms of G482 and A484 of the RBD. Lastly, a backbone-backbone interaction between TYR14 and S494 was observed in the Plantaricin W - RBD complex (See Figure 5 Panel A). In the Enterocin A-RBD complex, HIS3 of Bacteriocin formed two interactions with the S494 of RBD (Figure 5 Panel B). Interestingly, in the most populated state of Lactococcin Mmfii-RBD complex, we did not detect any polar interaction (See Figure 5 Panel C). However, VAL7 of Lactococcin Mmfii formed an interaction with N477 of RBD. Three polar interactions were observed in Salivaricin B-RBD complex, between HIS10-S494, GLU11-K493, and GLN18-N487 (First amino acid belongs to bacteriocin) (Figure 5 Panel D). For the Pediocin PA-1-RBD system, we observed four polar interactions. These interactions were observed between SER15-R498, SER15-Y501, and CYS44-R498 (two interactions) (Figure 5 Panel E). In terms of bacteriocin binding to RBD, S494 of RBD was found as a common interactor for Plantaricin W, Enterocin A, and Salivaricin B binding. Overall, the most populated states of the bacteriocin-RBD complexes reveal that the binding site of the RBD was occupied by all studied bacteriocins, and this will indicate that ACE2 will be prevented from approaching the RBD.

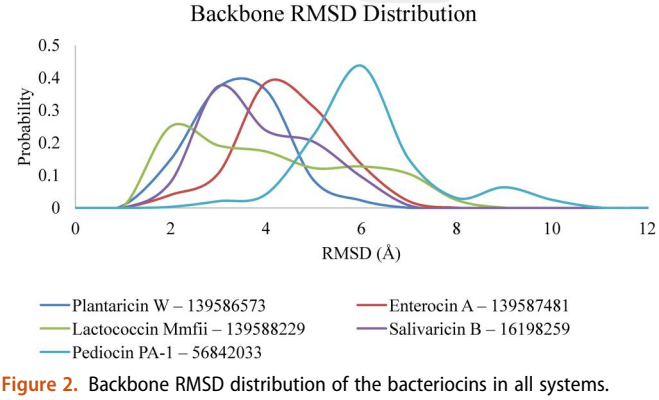


Figure 2. Backbone RMSD distribution of the bacteriocins in all systems.

## Discussion

The SARS-CoV-2 continues to threaten public health. The emergence of the Omicron (B.1.1.529.1 or BA.1) variant (Viana et al 2022) resulted in an urgent concern because of mutations in the S protein which resulted in evasiveness of antibodies (Iketani et al., 2022) and increased transmission ability which made it the predominant VOC in most places. Although the Omicron variant was reported to show alleviated pathogenicity owing to its modified cell tropism, it possesses greater resistance against vaccines, convalescent serum and most antibodies (Fan et al., 2022).

It was shown earlier that the four key mutations (namely: S477N, G496S, Q498R, and N501Y) of the Omicron RBD improved ACE2 binding. The effects of RBD mutations on antibody recognition were also investigated, particularly the S371L/S373P/S375F mutations, which drastically changed the local conformation of the resident loop and deactivated numerous class IV neutralizing antibodies (Lan et al., 2022).

Kim et al. 2022 quantified the interaction between Omicron RBD and ACE2 using a combined steered molecular dynamics (SMD) simulation and experimental microscale thermophoresis (MST) method (Kim et al., 2022). It was demonstrated that the Omicron variant has an improved RBD-ACE2 interface due to the mutations observed on the T478K, Q493K/R; and N501Y sites. The mutations also resulted in novel RBD-ACE2 interaction patterns, resembling the characteristics of previously dominant Alpha and Delta variants. It was also found that the mutations observed Omicron variant of RBD are linked with a fivefold increase in binding affinity to ACE2 relative to the wild type RBD strain. Overall outcomes of Kim et al., perhaps helps explaining the frequency of the Omicron variant in people world-wide, since greater forces of interaction or affinity for ACE2 likely facilitate

Table 2. Average MM/GBSA (in kcal/mol) and standard deviations for Bacteriocin-RBD systems.

| | MM/GBSA 139586573 | SD | MM/GBSA 139587481 | SD | MM/GBSA 139588229 | SD | MM/GBSA 16198259 | SD | MM/GBSA 56842033 | SD |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | −42,67 | 10,45 | −48,35 | 13,51 | −59,92 | 16,78 | −102,38 | 9,12 | −96,17 | 18,78 |
| 2 | −67,28 | 11,97 | −34,87 | 13,80 | −61,01 | 19,52 | −117,40 | 16,24 | −120,87 | 16,53 |
| 3 | −60,28 | 16,24 | −72,81 | 13,02 | −45,60 | 9,54 | −106,23 | 15,07 | −157,09 | 13,65 |
| 4 | −56,07 | 11,61 | −51,14 | 9,33 | −56,06 | 14,90 | −124,93 | 17,49 | −111,78 | 20,06 |
| 5 | −82,20 | 21,27 | −59,74 | 14,03 | −72,70 | 12,68 | −109,85 | 20,79 | −119,20 | 24,88 |
| 6 | −65,07 | 16,61 | −82,38 | 19,62 | −58,43 | 16,74 | −105,78 | 12,91 | −68,94 | 11,23 |
| 7 | −53,59 | 15,05 | −39,75 | 16,75 | −70,88 | 24,81 | −71,92 | 16,78 | −100,44 | 18,30 |
| 8 | −49,68 | 21,38 | −62,55 | 17,77 | −72,47 | 13,92 | −105,97 | 16,42 | −117,68 | 16,02 |
| average | −59,60 | 15,57 | −56,45 | 14,73 | −62,13 | 16,11 | −105,56 | 15,60 | −111,52 | 17,43 |

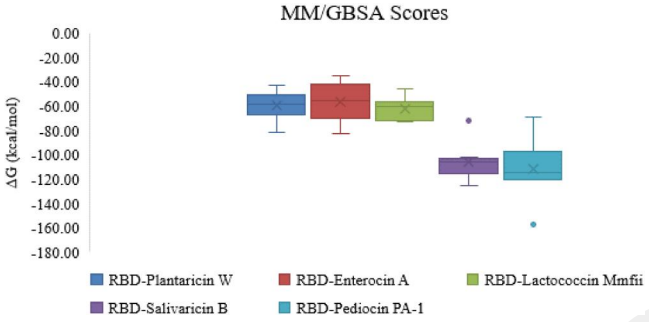


Figure 3. Box and Whisker plot for the MM/GBSA scores.

stronger internalization and viral binding, resulting in an increase in infectiousness (Fan et al., 2022).

The virus enters to the host cell by membrane fusion (Dong et al., 2020). The Omicron variant has a similar mechanism of infection. The affinity of ACE2 against Omicron's S protein is the crucial process in deciding viral infection capability. It was shown that the binding energies achieved for RBD of Omicron to ACE2 is 1.5 to 2.8 times greater than the wild type (X. Zhang et al., 2021)(Cui et al., 2022)(Cameroni et al., 2022). On the contrary, several reports showed the similar binding affinity of RBD to ACE2 between Omicron and Wild type (Han et al., 2022; Wu et al., 2022). ACE2 binding affinity for Omicron RBD is comparable to or lower than that of ACE2 binding affinity for the Delta variant (X. Zhang et al., 2021; Mannar et al., 2022; Wu et al., 2022)(Han et al., 2022). A single mutation in the RBD; N501Y, has reduced the binding capacity of the Omicron RBD to ACE2 to a significantly lower level than the Alpha variant (Cameroni et al., 2022)(Han et al., 2022). These results suggest that Omicron RBD's affinity for ACE2 is roughly in the middle of the range of WT and Delta RBD's. Omicron's interaction with ACE2 has been shown to be enhanced by the formation of new salt bridges or hydrogen bonds with ACE2's respective sites at S477N, T478K, Q493R, Q496S, and Q498R in addition to N501Y, K417N, and E484A, on the other hand, can significantly reduce polar contacts between Omicron and ACE2, therefore counteracting some of the positive effects of other mutations (Yin et al., 2022)(Cui et al., 2022)(Mannar et al., 2022)(Han et al., 2022). Overall, the Omicron RBD's receptor recognition and binding to ACE2 was not affected by mutations in the RBD, and the Omicron RBD was capable of binding to human ACE2 successfully for host cell entry. To penetrate the target cells, the Omicron S protein binds to human ACE2 or ACE2 orthologs from various animal species ( Hoffmann et al., 2022). Because of being potentially zoonotic, the Omicron variant might lead to the emergence of new and highly infective strains (Fan et al., 2022).

Even while new vaccines have made it possible to avoid COVID-19, there are no specific therapies to treat the disease. Attemps have been made by the scientists to find therapeutic compounds that block SARS-CoV-2 from entering the body. To combat the virus, antiviral medicines or immunomodulators can be used to block or regulate the host's cytokine storm (Garrett et al., 2022; Riediker et al., 2022). Finding promising therapeutic candidates has prompted the screening of a large number of chemicals, both synthetic and natural. Antiviral activity against Omicron variant has not been thoroughly studied in bacteriocins, however. Numerous biological effects, such as immunomodulatory impacts on the human body, are well-documented for these molecules. It is well-established that they have multiplebiological effects on the human body, including the ability to modulate the immune system (Chikindas et al., 1993). They could serve as an alternate source for the development of SARS-CoV-2 therapies because of their health-promoting characteristics (Akatsu, 2021; Chen et al., 1997). A few studies have suggested that bacteriocins may have a role in treating and preventing COVID-19 (Balmeh et al., 2021; Manna et al., 2021; Todorov et al., 2010).

As post-exposure prophylaxis, Wischmeyer et al., 2022 demonstrated that *Lactobacillus rhamnosus* GG was well-tolerated and associated with a delayed onset of COVID-19 infection, fewer symptoms, and alterations in gut microbiota composition. Lactobacilli probiotics have already been used in underdeveloped countries to lower non-COVID sepsis and infectious-morbidity, and this preliminary work might apprise the strategy to COVID-19 prophylaxis. Further *in vitro* studies are needed to better describe the efficacy of dietary supplements in preventing COVID-19 infection

A small number of bacteriocins derived from probiotic bacteria have been evaluated against SARS-CoV-2 utilizing *in silico* techniques. Present study evaluates 22 lactic acid bacteria-derived bacteriocins using molecular docking and MD simulations. The purpose of targeting the Omicron variant's RBD of SARS-CoV-2 is because this is the domain through which the virus interacts with host cells' ACE2 receptor and causes infection. The RBD domain of S protein plays a crucial role in the interaction between ACE2 (Wang et al., 2020).

In the current study, we conducted molecular docking experiments of mutant S viral protein against 22 lactic acid bacteria-derived bacteriocins as ligand library. This *in silico* screening was coupled with the validation utilizing MD

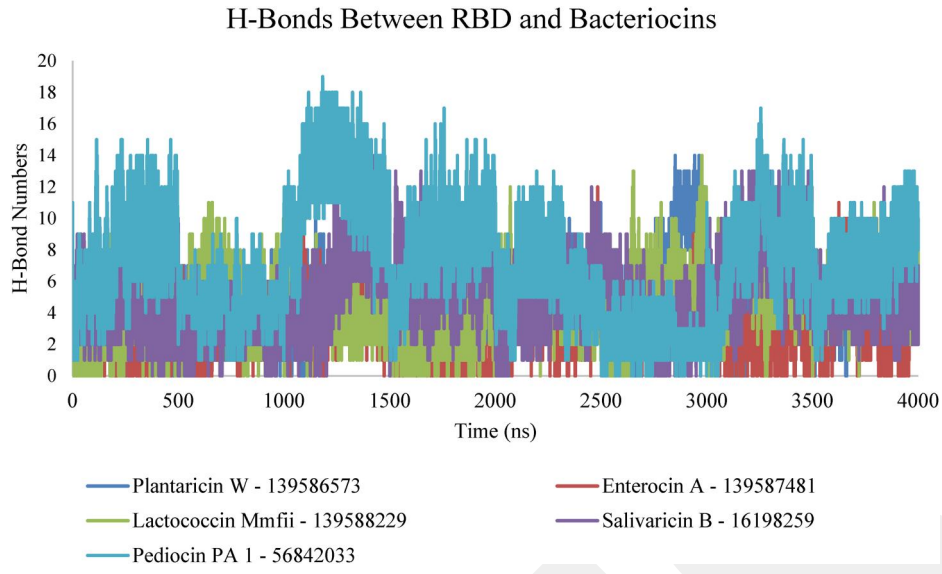


Figure 4. H-bond numbers for the RBD-Bacteriocin complexes.

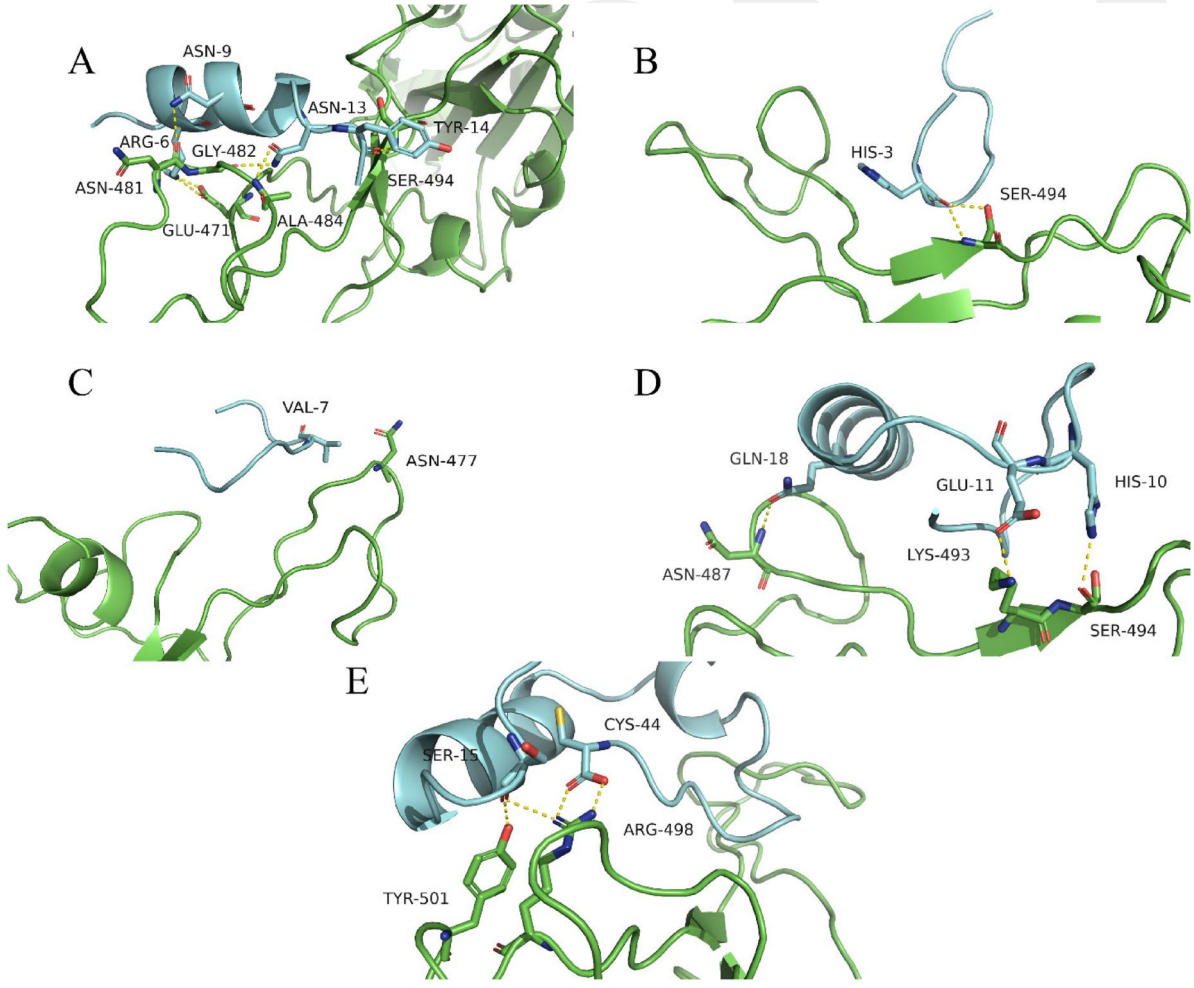


Figure 5. Constructed interactions of selected hit molecules with the RBD. Panel A: Plantaricin W – RBD; Panel B: Enterocin A-RBD; Panel C: Lactococcin Mmfii-RBD; Panel D: Salivaricin B-RBD; Panel E: Pediocin PA-1-RBD.

simulations to come up with potential inhibitors against the S protein of Omicron variant. Based on their binding affinity to the target macromolecule, Salivaricin B, Pediocin PA 1, Plantaricin W, Lactococcin mmfii, Enterocin A were selected as potential inhibitors. Based on the docking studies of these five leads, Salivaricin B was found to possess the lowest binding energy against the RBD of the Omicron variant. Salivaricin B is a 25 amino acid polycyclic peptide that is

produced by *Streptococcus salivarius* and belongs to the AII lantibiotics category (Barbour et al. 2016). Selected antibacterial activity against oral and upper respiratory tract microorganisms is possessed by salivaricins (Barbour et al., 2020). In particular, the broad-spectrum antimicrobial agent salivaricin B is a powerful antibacterial agent (Hyink et al., 2007). Except for salivaricin B, all salivaricins have a similar mechanism of action as pediocins. There are a number of proteins that produce pores in the membranes of pathogenic bacteria, such as salivaricin mmaye1 and salivaricin 9. Salivaricin B, on the other hand, does not cause the formation of pores in bacterial cells. It works by interfering with cell wall biosynthesis (Barbour et al., 2016). Salivaricin B has also been shown to have antiviral potential in the treatment of viral pharyngitis ( Di Pierro et al., 2014). Antiviral bacteriocins like peptide ST4V, enterocin CRL35, and enterocin ST5Ha have shown potential as preventive and therapeutic agents against COVID-19 (Lee and Paik, 2021). According to our computational analyses, the five most promising bacteriocins were identified as Salivaricin B, Pediocin PA 1, Plantaricin W, Lactococcin mmfii, Enterocin A. These bacteriocins are promising candidates for alternative treatment and prophylaxis of SARS-CoV-2 Omicron variant.

Molecular docking studies of bacteriocins against RBD of Omicron variant also showed that the second-best binding affinity score was achieved in Pediocin PA-1. Pediocin PA-1 is a type of non-lantibiotic bacteriocin that is effective against wide range of bacteria. It is produced by *Lactococcus lactis*, *Pediococcus acidilactici*, and *Pediococcus pentosaceous* and belongs to the class IIa bacteriocins (Barrett et al., 2007)(Barbour et al., 2020). Primarily, it demonstrates substantial biological action against *Listeria monocytogenes*, a foodborne pathogen and the culprit responsible for listeriosis (Horn et al., 1999; Rodríguez et al., 2002). Class IIa bacteriocins forms pores in the membrane of bacteria. This is the mechanism by which they exert their antibacterial effects (Chikindas et al., 1993). Because they are cationic in nature, bacteriocins target membrane receptors through electrostatic interactions (Chen et al., 1997). To our knowledge, pediocin PA-1 antiviral activity has not yet been studied. Todorov et al. (2010) showed that the pediocin-like bacteriocin has antiviral properties against herpes simplex virus, implying the antiviral potential of pediocin PA-1.

The third best predicted binding affinity score was achieved with Plantaricin W which is a two-peptide bacteriocin produced by *Lactobacillus plantarum* that inhibits a variety of Gram-positive bacteria, especially carries potent antimicrobial activity against *Listeria monocytogenes* (Holo et al., 2001)(Barbosa et al., 2016). Plantaricin Wα (consisting of 29 residues) and Plantaricin Wβ (consisting of 32 residues) had poor antibacterial activity but functioned synergistically. Chemical investigations revealed that both peptides are lantibiotics; however, Plantaricin Wα included two unmodified cysteines and one serine residue, while Plantaricin Wβ contained only one cysteine residue. It was determined that the Plantaricin W structural genes encode peptide precursors with sequence similarity to two other two-peptide lantibiotics, notably lacticin 3147 and staphylococcin C55. C-terminal conserved residues are predominantly cysteins, threonines, and serines that can participate in the creation of intramolecular thioether bonds. Each peptide has a core lanthionine and two overlapping thioether bridges adjacent to its C-terminus, according to a proposed structural model (Holo et al., 2001). Anwar et al. (2021) reported the antiviral activity of Plantaricin W against the wild type of SARS-CoV-2; however, the activity of Plantaricin W against the Omicron variant is being demonstrated in the present study.

The fourth best binding affinity score was achieved with Lactococcin MMFII which is a Class II bacteriocin biosynthesized by *Lactococcus lactis* MMFII that was isolated from a Tunisian dairy product. It shows heat stability and pH resistance which the latter implies that Lactococcin MMFII could tolerate the harsh gastric digestive conditions. Its amino acid sequence showed a 37-amino acid peptide comprised of two cysteine residues and a molecular mass of 4142.6 Da. Lactococcin MMFII has the N-terminal YGNGV consensus motif and is inhibitory against *Listeria monocytogenes* (Ferchichi et al., 2001). According to Balmeh et al., 2021, glycocin F from *Lactococcus lactis* and lactococcine G from *Lactobacillus plantarum* are the peptides conferring high affinity against SARS-CoV-2 S, N, and 3CL protease. Lactococcin G was also found to possess a high binding affinity to RdRp protein. It was reported that optimizing glycocin F and lactococcin G can turn these two peptides into safe therapeutics against SARS-CoV-2 (Balmeh et al., 2021). The potential antiviral activity of Lactococcin MMFII is being shown in the current study against the RBD of SARS-CoV-2 Omicron variant.

Enterocin A is the fifth tightest binder against the Omicron variant RBD which was isolated from *Enterococcus faecium*. Enterocin is composed of 47 amino acid residues with a molecular weight of 4,829 Da . The amino acid sequence of enterocin A is highly similar to that of pediocin-like bacteriocins isolated from the genera Lactobacillus, Pediococcus, Leuconostoc, and Carnobacterium, with which it shares significant homology (Aymerich et al., 1996). Enterocin A has an isoelectric point of around 10, and likely a cyclic peptide. Thus, owing to the peptide cyclic structure and outstanding heat stability, it is a viable candidate for combatting the bacterial pathogens (Franz et al., 2003)(Satish Kumar et al., 2011; Fathizadeh et al., 2020). Due to the fact that Enterococci carry resistance genes and possible virulence factors, the synthesis of enterocins by a safe bacterial host has recently garnered considerable interest (Gutiérrez et al., 2006).

Our MM/GBSA analyses showed that the tightest binder among all studied bacteriocins was the Pediocin PA 1. This data also supported with number of H-bonds between RBD and Pediocin PA 1, where the average number of hydrogen bonds was $7.05 \pm 3.42$. The second system was Salivaricin B according to MM/GBSA results, where the number of H-bonds was found as $4.90 \pm 2.42$. The MM/GBSA binding energies of the other 3 systems (Lactococcin Mmfii, Plantaricin W, and Enterocin A) and the hydrogen bond numbers they had at the interface were found to be similar to each other.

In the most populated structure of RBD—Salivaricin B complex (Figure 5 and Figure S12, Panel D), three key interactions were observed between N487-GLN18, K493-GLU11, and S494-HIS10. N487 and K493 residues of RBD identified as key positions for effective binding and inhibition of SARS-CoV-2 (Barh et al., 2020). Similarly, for the RBD—Pediocin PA 1 complex (Figure 5 and Figure S12, Panel E), SER15 and CYS44 formed four interactions with R498 and Y501, where the latter two residues of RBD reported to restore ACE2 binding compared to K417N variant (Li et al., 2022 ). In RBD—Plantaricin W complex (Figure 5 and Figure S12, Panel A), E471-ARG6, N481-ASN9, G482-ASN13, A484-ASN13, and S494-TYR14 interactions were observed, where in recent study A484 and S494 were found to contribute peptide binding with multiple interactions (Sakib et al., 2021 ). In RBD—Enterocin A and RBD—Lactococcin Mmfii complexes, S494-HIS3 and N477-VAL7 interactions were observed in the most populated cluster structures (Figure 5 and Figure S12, Panel B and Panel C, respectively). Importance of S477 was highlighted by Singh et. al. and they showed that mutation on this position increased the binding affinity to the hACE2 (Singh et al., 2021). Overall, our binding analyses of Bacteriocins to RBD omicron variant concluded that, all interactions that we observed at the binding interface have the ability to interact with RBD and prevent ACE2 binding.

Bacteriocins affinities against the RBD of Omicron variant were also evaluated across the lead compounds of Salivaricin B, Pediocin PA 1, Plantaricin W, Lactococcin mmfii, and Enterocin A. Based on MM/GBSA binding free energies, the most stable bacteriocin appeared to be Pediocin PA-1 and this observation is also supported by hydrogen bound counts. Pediocin PA 1, which was found to be the best binding bacteriocin in our previous study (i.e., where WT and beta variant were considered ), was also observed as the most tightly binding system in the Omicron variant. The second best average MM/GBSA-scored compound was found to be Salivaricin B which is followed by Lactococcin mmfii, Plantaricin W, and Enterocin A. Therefore, promising binding free energy results were achieved across five lead compounds out of 22 bacteriocins tested in the present study.

## Conclusions

Protein-protein docking for 22 bacteriocins produced by lactic acid bacteria against mutated S protein of the SARS-CoV-2 Omicron variant revealed that Salivaricin B, Pediocin PA 1, Plantaricin W, Lactococcin mmfii and Enterocin A had strong predicted binding affinities toward the mutant S protein. MD simulations and binding free energy analyses confirmed the strong binding of bacteriocins to the mutant receptor-binding domain (RBD) of the spike protein. The lead peptides arised from protein-protein in silico docking study were produced by GRAS status lactic acid bacteria apart from Enterocin A which is being produced by pathogenic Enterococci. Based on this, it is suggested that these four bacteriocins could potentially be used as therapeutic options against the Omicron variant of SARS-CoV-2. This would require further research, including in vitro and in vivo studies and clinical trials, to be carried out.

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