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Unlocking the antibacterial potential of bacteriocin peptides: A subtractive proteomics approach to target food spoiling *Serratia marcescens*

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Authors' Contribution | **Yousafi, Q.** conceptualized study, designed methodology, interpreted results & **D. Fatima** executed methodology and performed computational analysis.

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ABSTRACT

Food deterioration is a serious environmental issue as well as a major concern of the food industry. Bacteriocins, a natural food preservative, can help to resolve these issues. The peptide sequences of 111 bacteriocins were obtained from UniProt. The physicochemical properties and immunogenicity of peptides were identified. Final 55 non immunogenic antimicrobial peptides were selected. Full proteome of *Serratia marcescens,* containing 4,706 proteins, was downloaded from UniProt. The subtractive proteomic approaches were used to select target pathogenic proteins. Only seventy-two proteins from outer membrane, cytoplasmic membrane, extracellular and periplasm were selected for further analysis. Only two *S. marcescens* proteins, UPI0002AF278A and UPI0003C9BA85, were docked with 55 bacteriocins. Five protein-bacteriocin complexes were subjected to MD simulation by using Amber tool. Three complexes, UPI0003C9BA85-P36504, UPI0003C9BA85-P80925m and UPI0003C9BA85-P86394m were found stable during 50ns run of MD simulation. These three bacteriocins are suggested for further laboratory testing to be used as food preservatives.

Keywords: Food preservatives, peptide docking, molecular dynamic simulation, subcellular localization.

INTRODUCTION: Food is naturally organic and primarily made up of compounds like water, proteins, carbohydrates, fats, minerals, and vitamins. These days, people are worried about their consumption and how it may impact their health. Organic and fresh food are healthier than those containing synthetic chemicals known to cause cancer and inflammations. The demand for fresh food, free of preservatives, is increasing day by day due their health benefits. Food degradation refers to the metabolic process that leads to the deterioration of food, causing it to lose its sensory qualities and become unsuitable for human consumption. This degradation can manifest as changes in texture, aroma, flavor or visual appearance, rendering the food unappealing and inedible. It is important to note that even though spoiled foods may still be safe to eat, the variations in sensory qualities often make them unacceptable for consumption (Rawat, 2015). A number of factors, such as heating, freezing, pollutants and microorganisms, can cause food to become contaminated, leading to consumers dissatisfaction and non-preference (Nerín *et al.*, 2016). Various chemical and physical changes had to be made to foods during preservation. The main bioactive decay of foods is characterized by water loss, which result in physical destruction, off-flavor and decrease nutritional quality (Kong and Singh, 2016). Microbes are largely to blame for food contamination and the loss of a sizable portion of food. Food that has been contaminated by microorganisms such as bacteria, viruses, mold, fungi and toxins is often described as having a high microbiological burden or microbial contamination. Globally, there is a major public health concern regarding food-borne illnesses brought on by *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Listeria monocytogenes (*Juneja *et al.,* 2012*).*The majority of food processing operations have as their main goal the restoration of food and their biggest challenge is ensuring the security and quality of their processed goods. Over the past 3 decades, the use of food additives has grown significantly, reaching over 200,000 tons annually. Therefore, it has been anticipated that each individual now takes up an average of 8 to 10 pounds of food additives per year, with some possibly eating significantly more, as about 75% of the Western diet is made up of different packaged foods (El-Samragy, 2012). Sodium nitrite is a food additive, added to lunch meats, hams, sausages, hot dogs, and bacon, to stop botulism. Sodium nitrite performs the crucial task of preventing botulism, but it can also interact with proteins cause cancer due to production of N-nitrosamines when food is cooked at high temperatures (Organization, 1988). It has been discovered that the frequently used sodium benzoate can keep bottled tomato sauce fresh for an additional 40 weeks without compromising quality. However, when merged with vitamin C, it can produce the carcinogen benzene. Billions and billions of microbes are thought to exist both inside and outside of our bodies. Microbes may be able to prevent neighboring pathogenic organisms from spreading. Bacteriocins are ribosomal formed peptides with antimicrobial effects on bacteria that are either intimately related or unrelated to them (Ng *et al.*, 2020a). To eliminate or suppress the growth of various microorganisms as a means of competition and survival in the microbial population, they are either bactericidal or bacteriostatic (Darbandi *et al.*, 2022). The mechanism of action, inhibitory spectrum, molecular mass, biochemical processes and genetic make-up of the bacteriocins produced by each species of bacteria differ significantly from one another. Since almost all bacteria generate at least one bacteriocin, there are many of these antimicrobial peptides, but most of them are still unknown. Some strains of E. coli prevent the growth of other strains of the same species was reported in 1925 and those inhibiting substance was later identified as bacteriocin (Gradisteanu Pircalabioru *et al.*, 2021). The food companies primarily use the commercially available bacteriocin, which appears to contain food-grade fermengates. Strategies for identifying bacteriocins in genomic and meta-genomic data have been devised (Talluri, 2019).

Bacteriocins have been divided into different groups according to their physical characteristics, consistency, chemical composition, molecular mass, mechanism of action and the type of source organism. Bacteriocins, which are frequently used in the fermentation of products like sausage, vegetables and dairy foods, have the ability to prevent the growth of bacteria that cause spoilage of food or pathogenicity (Khan *et al.*, 2010). Bio preservation is the method of keeping food for a long time using natural substances like bacteriocins without degrading its nutritional value (Singh, 2018). The use of more natural preservatives rather than chemical preservatives has been strongly demanded by consumers due to their desire for quicker, nutritious and fully prepared products. To solve this issue and meet the need for food preservation, bacteriocin-producing microorganisms attracted the attention of microbiologists all over the world. Due to its temperature resistance, wider pH tolerance and proteolytic activity, bacteriocin is used as a food preservative. It is also non-toxic, flavorless, and colorless (Darbandi *et al.*, 2022). Serratia's participation in the spoilage of foods (such as eggs, butter, milk, coconut, and bread) and the hyperpigmentation of cheeses is another significant trait in the discipline of food microbiology. Additionally, it leads to the formation of meat odours and forestation. Milk contaminated with Serratia may contaminate dairy products (Robinson, 2014). *Serratia sp*. is a member of the family Enterobacteriaceae and a Gram-negative bacterium (Barman *et al.*, 2020). *Serratia* sp. can persist in foods like smoked and dried fish that are inappropriate for the growth of other bacteria. Some *Serratia* sp. strains can grow at a mean minimum temperature of 1.7 C in beef, while others can withstand pressure during the handling of ground chicken (Robinson, 2014). The most frequently found specie of *Serratia* in food spoilage is *S. marcescens.* This food spoiling bacteria is the main target of our research. The aim of this study is the Identification of novel targets in food spoiling bacteria through subtractive proteomics approach. Identification of bacteriocins that can inhibit the function of essential proteins in food spoiling bacteria. Bacteriocins are naturally occurring food additives produced by a variety of bacteria to kill other bacteria (Talluri, 2019). Antibiotics or food preservatives are added to foods to improve shelf life and prevent microbial development and possible contamination. Bacteriocins can also be used instead of antibiotics

because some bacteria are resistant to antibiotics. Bacteriocins are the perfect candidate for killing these pathogenic bacteria.

OBJECTIVES: The objectives of this study werethe identification of novel targets in food spoiling bacteria and their potetntial inhibitors (bacteriocines) through subtractive proteomics approach

MATERIALS AND METHODS: Stage I: Bacteriocin peptides dataset mining and screening: Amino acid sequences bacteriocins were collected from the UniProt (https://www.uniprot.org/) database, a publicly available database of information on the structure and function of proteins. The immunogenicity of selected peptides was determined by using CD4 T cell immunogenicity prediction (http://tools.iedb.org/CD4episcore/) online program by IEDB. The prediction is performed by using NNalign algorithm. Non-immunogenic peptides were selected for further analysis (Dhanda *et al.*, 2019). I-Mutant (https://folding.biofold.org/i-mutant/i-mutant2.0.html) has been used to incorporate point mutation in peptides. It uses SVM-based method for automatically predicting how single point mutations affect protein expression (Capriotti et al., 2005). Antimicrobial peptides (AMPs), crucial elements of the innate immune system, have been shown to be efficient against pathogenic proteins. The web service AntiBP2 (http://crdd.osdd.net/raghava/antibp2/) was used to evaluate the anti-microbial potency of selected peptides (bacteriocins) (Meher *et al.*, 2017). Numerous antibacterial peptides have been successfully authorized as drugs by the FDA over the recent decades, sparking interest in these antibacterial peptides (Lata *et al.*, 2010). Three dimensional (3D) of the bacteriocins were predicted by trRosetta (https://yanglab.nankai.edu.cn/trRosetta/) (Du *et al.*, 2021). The predicted 3D structures were submitted to SAVES v6.0 server for quality check. FoldX and I-Mutant (https://folding.biofold.org/i-mutant/i-mutant2.0.html) were used to determine the effect of mutation on the stability of protein structure (Schymkowitz *et al.*, 2005). ProtParam (https://web.expasy.org/protparam/) was used to calculate the physicochemical properties of peptides such as molecular weight, Instability index, GRAVY (Grand average of hydropathicity), theoretical PI, extinction coefficient and half-life of the peptides under study (Gasteiger *et al.*, 2005; Garg *et al.*, 2016). ProtParam from Expasy (Gasteiger *et al.*, 2005) server is a reliable algorithm to compute physico-chemical properties. Instability Index and half-life of peptides reflect the bioavailability of the peptides. The threshold for protein stability was kept Instability Index< 40 and half-life< 30hours.

Stage II: Food spoiling bacterial protein dataset mining and preparations: The whole proteome of *S. marcescens*: Strain Db11 (UP000037482), containing 4,706 proteins, was downloaded from the UniProt (https://www.uniprot.org/). The Paralogs in the proteome were removed by CD-HIT (Cluster Database at High Identity with Tolerance) (https://www.bioinformatics.org/cd-hit/) suite. This tool uses a clustering algorithm, for removal of redundant sequences (Li and Godzik, 2006). Essential proteins play an important role in the survival of an organism (Zhang and Ren, 2015). These essential proteins can be pathogenic and virulent and by targeting these proteins, pathogenic bacteria can be easily destructed. The dataset of bacterial essential protein sequences was downloaded from the web-database NetGenes (https://rbc-dsai-iitm.github.io/NetGenes/).Virulent proteins play an important role in microbial pathogen as they mediate many pathogenic pathways in host leading towards severe diseases and can serve as best drug candidates (Asad *et al.*, 2018). VFDB (virulence factor database)(http://www.mgc.ac.cn/VFs/) (Li and Godzik, 2006) was used to identify the virulent proteins in proteome. The physiochemical properties of the selected were predicted by ProtParam as mentioned in stage I. The amino acid sequence of the proteins of *S. marcescens* were subjected to PSORTb (https://www.psort.org/psortb/) (Yu *et al.*, 2010) for prediction their subcellular localization. The proteins localized only in cytoplasmic membrane, periplasm, extracellular and outer membrane were selected for further study. The 3D structures of selected proteins were predicted using Geno3D (http://geno3d-pbil.ibcp.fr). Geno3D (Combet *et al.*, 2002) is an automatic web server for protein molecular modelling.

Stage III: Protein-peptide docking and molecular dynamic simulation: Protein (pathogen)-peptide (bacteriocins) docking was performed by PyDock (https://life.bsc.es/pid/pydock/) to identify residues involved in interactions between proteins (pathogen) and peptides (bacteriocins). It is a protein-protein docking algorithm that uses electrostatics, desolvation energy and a limited van der Waals contribution to score rigid-body docking poses. The top five docked complexes, with the lowest binding energy were selected to perform molecular dynamic (MD) simulations. Molecular Dynamic simulations helped in providing detailed information about protein-peptide complex perturbations and conformational charges.The first step was system preparation which includes preparation of input files. Amber tools were used for this purpose to prepare files for final production run. A large set of molecular mechanical force fields for simulations were applied through xleap program provided in Amber package. The input files were prepared by leaprc *ff14SB* force field. Two files were subjected to the system, first was coordinates file (inpcrd) that contains the information about Cartesian coordinates of 3D structures of protein and peptide: second was topology file (prmtop) containing information about atoms, bonds, angles and molecules present. *TIP3P* water model was used to prepare all systems. The next step in system preparation was loading the PDB file of complex (protein-peptide) then charge was checked on the system. The final step of simulation was to run simulations in the production phase for 50nano seconds. During the production run conformational changes that took place after every 2fs time step were saved in the form of trajectories in DCD file, produced by NAMD.

RESULTS: Stage I: bacteriocin peptides dataset mining and screening: Total 172 bacteriocin sequences were retrieved but only 72 peptides sequences having length ≤50 were selected for further (table 1). Out of 72 selected peptides only 56 peptides were found non-immunogenic (table 1).

No	UniProt-ID	No	UniProt-ID
1	A0A0B7MF59	29	P36502
2	A0A0E2P8G1	30	P36503
3	A0A0P0C3P7	31	P36504
4	A0A0U0CP47	32	P36961
5	A0A1E9GG72	33	P36962
6	A0A1S9ZCG0	34	P42723
7	A0A4U1LBM3	35	P80214
8	A0A6G2DJX7	36	P80666
9	A0A6I1TUZ4	37	P80925
10	A0A150NII9	38	P80959
11	B1I839	39	P81052
12	B3A0N4	40	P81053
13	C0HJC0	41	P83002
14	C0HL39(n)	42	P83378
15	C0HLU4	43	P83674
16	C1CAF2	44	P84886
17	C1CBU6	45	P84962
18	C6FX52	46	P85065
19	D3VML5	47	P85148
20	E0Q3I4	48	P85833
21	E8K1W4	49	P85876
22	E9FQC5	50	P86291
23	J1P1V9	51	P86393
24	O07623	52	P86394
25	P0DQM5	53	P86395
26	P01547	54	Q48501
27	P02987	55	S7Z987
28	P36499	56	V8IIJ8

Table 1: List of Non-immunogenic bacteriocin peptides retrieved from UniProt.

Point mutation was introduced in 56 selected peptides (table 2). Only 55 peptides out of 112 (56 wild type+56 mutated) showing antimicrobial properties were selected for further analysis rest were discarded. Moreover, mutant (M) of five peptides exhibited AMP while their wild type (WT) were non antimicrobial. Predicted and refined three dimensional (3D) structures 55 antimicrobial peptides are presented in table 2. The structural stability of 11 peptides was found to be increased after point mutation (table 2). Various physicochemical parameters of proteins such as amino acid composition, extinction coefficient (Garg *et al.*, 2016), instability index, grand average of hydropathicity (GRAVY), aliphatic index, theoretical PI, atomic composition and molecular weight allows us to understand the stability, activity and nature of protein.

Only 4 proteins (P01547, P01547m, P36502, and P36502m) were unstable having instability index> 40 (table 3). Twelve peptides which showed positive GRAVY values were hydrophobic while 4 peptides were hydrophilic in nature with negative GRAVY values. The estimated half-life of 22 peptides was found ≥30 which were discarded.

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No.	ID	Position	WT*	NEW	Stability
1	A0A0B7MF59	9	R	H	Decrease
2	A0A0E2P8G1	14	K	T	Decrease
3	A0A0P0C3P7	23	V	Y	Decrease
4	A0A0U0CP47	28	G	W	Decrease
5	A0A1E9GG72	17	Q	A	Decrease
6	A0A1S9ZCG0	2	M	V	Decrease
7	A0A4U1LBM3	12	S	F	Increase
8	A0A6G2DJX7	7	N	F	Increase
9	A0A6I1TUZ4	34	A	I	Decrease
10	A0A150NII9	30	G	M	Increase
11	B1I839	3	K	I	Decrease
12	B3A0N4	28	N	F	Decrease
13	C0HJC0	22	I	H	Decrease
14	C0HL39	3	P	F	Decrease
15	C0HLU4	15	Q	A	Decrease
16	C1CAF2	19	I	F	Decrease
17	C1CBU6	35	G	F	Decrease
18	C6FX52	15	E	K	Increase
19	D3VML5	38	S	F	Decrease
20	E0Q3I4	21	G	R	Decrease
21	E8K1W4	24	G	A	Decrease
22	E9FQC5	16	E	F	Increase
23	J1P1V9	4	D	H	Decrease
24	O07623	28	P	K	Decrease
25	P0DQM5	12	T	F	Decrease
26	P01547	5	C	R	Decrease
27	P02987	23	Q	D	Decrease
28	P36499	49	C	F	Decrease
29	P36502	9	P	Y	Decrease
30	P36503	3	N	L	Increase
31	P36504	11	T	L	Decrease
32	P36961	11	G	L	Decrease
33	P36962	8	W	S	Decrease
34	P42723	23	A	C	Decrease
35	P80214	37	K	F	Increase
36	P80666	10	G	I	Decrease
37	P80925	2	Y	L	Increase
38	P80959	28	K	Q	Decrease
39	P81052	21	Q	F	Decrease
40	P81053	3	Y	L	Decrease
41	P83002	31	N	F	Increase
42	P83378	5	K	S	Increase
43	P83674	23	G	K	Decrease
44	P84886	2	Y	L	Decrease
45	P84962	4	Y	A	Decrease
46	P85065	14	P	F	Decrease
47	P85148	21	V	C	Decrease
48	P85833	17	K	A	Decrease
49	P85876	31	I	F	Decrease
50	P86291	34	D	F	Decrease
51	P86393	4	Y	L	Decrease
52	P86394	21	I	S	Increase
53	P86395	25	F	I	Decrease
54	Q48501	19	G	F	Decrease
55	S7Z987	44	K	Y	Decrease
56	V8IIJ8	2	M	L	Decrease

Table 2: Point mutation introduced in bacteriocin peptides and its effect on structural stability.

Stage II: Food spoiling bacterial protein dataset mining: The full proteome of food spoiling bacterial specie *Serratia marcescens* strain Db11 (UP000018979) was retrieved from UniProt kb. The proteome was comprised of total 4,706 proteins. CD-HIT (Cluster Database at High Identity with Tolerance) suite was used for the removal of paralogs and 3, 870 genes were retrieved. Out of these screened proteins 2,711 were identified as essential protein by NetGenes. The sequences of selected essential proteins were subjected to VFDB for BLASTp to identify virulent proteins using threshold of bit score≥ 350. The virulent proteins (111) were selected for further study (table 3). Subcellular localization of target proteins was predicted through online web servers PSORTb. Total 78 target proteins from 4 localizations i.e., Outer membrane (14), Cytoplasmic Membrane (20), Extracellular membrane (10) and Periplasmic membrane (34) were selected. Structure prediction of UPI0002AF278A and UPI0003C9BA85 was done by Geno3D. The validation of 3D structures was performed to check the reliability and quality of structures by using SAVES 6.0 server and refined structures are shown in figure 1.

Stage III: Protein-peptide docking and MD simulations: An online tool PYDOCK was used for the identification of important residues involve in interactions between proteins (food spoiling bacteria) and peptides (bacteriocins). The docking for 2 selected proteins, UPI0002AF278A and UPI0003C9BA85 of *S. marcescens* and 55 peptides (bacteriocins) was performed by PYDOCK server (tables S5-6). Molecular Dynamic simulations for UPI0003C9BA85-P36504 (figure 2a), UPI0003C9BA85-P80925m (figure 2b) and UPI0003C9BA85-P86394m (figure 2c), was performed to study the structural and conformational changes occurred in proteins and peptides.

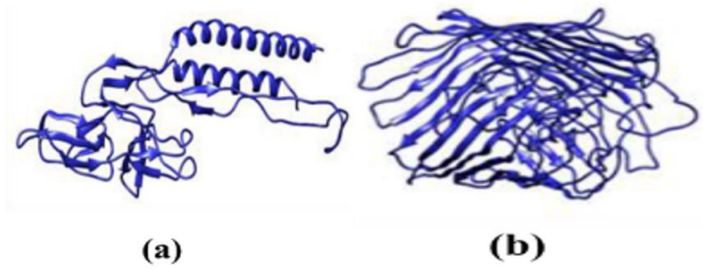

Figure 1: 3D structure of *S. marcescens* pathogenic proteins (a) UPI0002AF278A (b) UPI0003C9BA85.

No.	UniProt ID	Molecular Weight	Theoretical PI	Instability index	Extinction coefficient	Estimated half-life	GRAVY
1	A0A0B7MF59	5556.56	9.73	28.70	0.536	30	-0.282
2	A0A0B7MF59m	5537.52	9.52	34.50	0.538	30	-0.256
3	A0A1S9ZCG0	5529.49	9.52	28.70	0.539	30	-0.330
4	A0A1S9ZCG0m	5497.43	9.52	28.70	0.542	30	-0.284
5	A0A6I1TUZ4m	5569.65	9.73	23.20	0.535	30	-0.120
6	B3A0N4	4448.93	9.19	23.0	1.934	1.3	-0.433
7	B3A0N4m	4482.00	9.19	19.50	1.92	1.3	-0.286
8	C0HJC0	3497.97	11.47	3.00	2.424	30	-0.767
9	C0HL39(n)m	2934.39	4.03	30.90	0.043	5.5	0.617
10	C1CBU6	5556.56	9.73	28.70	0.536	30	-0.282
11	J1P1V9m	5579.6	9.73	28.68	0.534	30	-0.276
12	O07623m	4356.15	5.96	20.45	1.291	30	0.633
13	P01547	4680.21	3.80	41.00	2.555	4.4	0.249
14	P01547m	4733.26	5.82	47.80	2.500	4.4	0.093
15	P36502	2063.35	7.96	67.59	0.061	1.2	-0.168
16	P36502m	2129.41	7.95	63.12	0.758	1.2	-0.153
17	P36503	2007.19	5.81	32.61	3.545	1.2	-0.511
18	P36503m	2006.25	5.81	32.61	3.546	1.2	-0.126
19	P36504	2069.35	7.95	20.05	0.060	1.2	-0.189
20	P36504m	2081.41	7.95	73.56	0.060	1.2	0.047
21	P36961	4345.93	10.16	14.44	2.874	30	-0.841
22	P36961m	4402.03	10.16	14.32	2.837	30	-0.733
23	P42723	4406.23	10.11	10.70	0.000	30	-0.281
24	P80214	5457.65	10.48	13.82	2.289	30	-0.475
25	P80214m	5476.65	10.43	10.65	2.281	30	-0.335
26	P80666	2424.76	8.52	13.67	2.986	1.1	-0.014
27	P80666m	2480.87	8.52	13.67	2.918	1.1	0.209
28	P80925	4289.81	9.45	26.27	3.288	1.3	-0.253
29	P80925m	4239.79	9.51	10.42	2.975	1.3	-0.135
30	P80959	3357.92	10.00	10.82	0.887	30	-0.387
31	P80959m	3357.88	9.82	21.20	0.887	30	-0.374
32	P81053	4598.04	8.79	18.09	4.264	1.3	-0.665
33	P81053m	4548.02	8.82	20.07	3.983	1.3	-0.547
34	P83002	4144.64	6.43	27.62	3.403	7.2	-0.535
35	P83002m	4177.72	6.43	33.60	3.376	7.2	-0.365
36	P83378	2545.04	9.41	30.55	1.171	4.4	0.100
37	P83378m	2503.94	8.47	34.41	1.190	4.4	0.241
38	P84886	3024.33	8.07	33.88	1.027	4.4	-0.360
39	P84886m	2974.32	8.08	36.19	0.543	4.4	-0.190
40	P84962	4525.21	8.8	3.74	3.474	7.2	-0.188
41	P84962m	4433.12	8.82	0.87	3.210	7.2	-0.116
42	P85065m	2404.70	5.47	42.30	2.391	100	0.517
43	P85148	4125.71	8.29	3.85	4.451	7.2	0.164
44	P85148m	4129.72	8.22	1.48	4.447	7.2	0.121
45	P85876	3932.40	8.79	21.01	2.188	4.4	-0.221
46	P85876m	4000.43	8.79	25.48	2.151	4.4	-0.311
47	P86291	4602.17	6.44	23.83	3.065	7.2	-0.590
48	P86291m	4634.26	7.71	23.83	3.044	7.2	-0.437
49	P86393	4630.18	9.31	27.94	4.851	4.4	-0.908
50	P86393m	4580.16	9.36	26.95	4.578	4.4	-0.777
51	P86394	3629.08	9.39	20.75	1.961	100	0.046
52	P86394m	3603.00	9.39	9.89	1.975	100	-0.106
53	S7Z987	5511.46	9.52	27.56	0.541	30	-0.278
54	S7Z987m	5546.46	9.25	26.05	0.806	30	-0.226
55	V8IIJ8m	5473.47	9.06	26.20	0.567	30	-0.048

Table 3: Physicochemical properties of bacteriocin peptides.

Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Radius of gyration (Rg) were calculated and their plots were generated. RMSD plots helps to analyze the stability of a protein or complex with respect to time and also indicates the conformational changes and displacements occur in them. MD simulations were performed to study the dynamic behavior of protein alone and in protein-peptide complex.

The figure 5 showed the RMSD plots of UPI0003C9BA85 (*Serratia marcescens*) and its three complexes with starting 1000 frames

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(3ns) excluded. In this plot of UPI0003C9BA85 (blue), we can notice the average RMSD value ranges between 1 and 1.5 Å with almost no deviation. This value indicates the stability of UPI0003C9BA85 protein throughout the simulations. The RMSD plot of UPI0003C9BA85-P36504 complex (figure 3b) shows an average value of RMSD from 1 to 1.8 Å with almost no deviation. The RMSD plot of UPI0003C9BA85-P80925m complex (figure 3c) ranges from 1 to 1.5 Å and where it shows deviation from 6 to 8 ns and 16 to 18 ns and then it becomes stable.

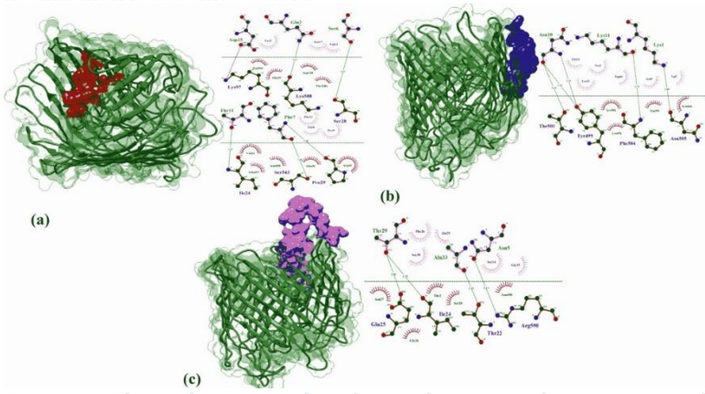

Figure 2: Three dimensional and two dimensional interaction of complexes selected for MD Simulation. (a) UPI0003C9BA85-P36504 (b) UPI0003C9BA85-P80925m (c) UPI0003C9BA85-P86394m

A little deviation is also observed at 20 and 23 ns and then seems to be stable throughout the simulation. The RMSD plot of UPI0003C9BA85-P86394m complex (figure 3d) ranges from 1 to 2 Å. It is observed that the RMSD value slightly increasing with the passage of time from 12 to 20 ns but after that it become stable up to 50 ns.

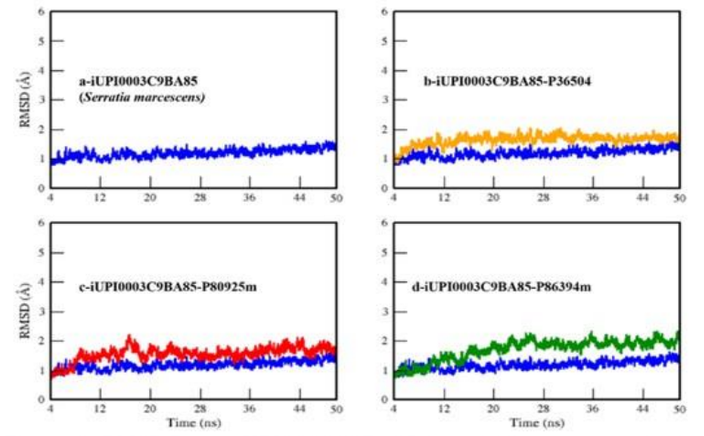

Figure 3: RMSD Plot of UPI0003C9BA85 (blue) (*S. marcescens*) with its three peptides, a- UPI0003C9BA85, b- UPI0003C9BA85- P36504 complex, c-UPI0003C9BA85-P80925m complex, d-UPI0003C9BA85 -P86394m

Overall we can say that peptides like P36504 and P80925m and P86394m did not cause any large structural changes throughout simulations. All the complexes show maximum stability throughout simulations of 50 ns. Root Mean Square Fluctuation was calculated to analyze the atomic fluctuations in residues and to identify the fluctuating regions in protein and complexes. Lower RMSF values indicate stable residues whereas high RMSF value shows flexibility and fluctuations of the respective residues. The figure 6 shows the RMSF plot of UPI0003C9BA85 (*S. marcescens*) and its three complexes. No major peak was observed for RMSF of UPI0003C9BA85 (figure 4a). RMSF graphs of UPI0003C9BA85-P36504 (figure 4b), UPI0003C9BA85-P80925m (figure 4c), and UPI0003C9BA85-P86394m (figure 4d) were compared and all of them show almost same pattern where no higher peaks were for all three complexes the average value of RMSF was ranges from 1 to 2 Å. present. The figure 5 showed the Rg plot of UPI0003C9BA85 (*S. marcescens*) and its three complexes. It has been observed that the value of ROG for UPI0003C9BA85-P36504 (figure 5a), UPI0003C9BA85-P80925m (figure 5b), and UPI0003C9BA85-P86394m (figure 5c) complexes remain steady indicating that the complex remains stable throughout the 50 ns simulations and no unfolding event is observed.

DISCUSSIONS: Food deterioration during storage is a major environmental problem and is a major concern of food industry. Food spoilage can be defined as "any sensory change (tactile, visual, olfactory or flavor)" which the consumer considers to be unacceptable. Spoilage may occur at any stage along food chain.

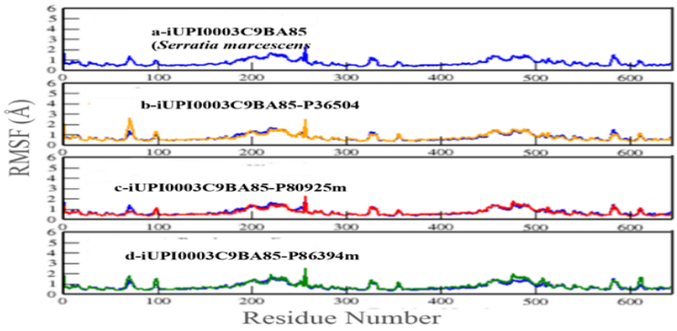

Figure 4: RMSF Plot of UPI0003C9BA85 (blue) (*S. marcescens*) with its three peptides, a- UPI0003C9BA85, b- UPI0003C9BA85- P36504 complex, c-UPI0003C9BA85-P80925m complex, d-UPI0003C9BA85-P86394m.

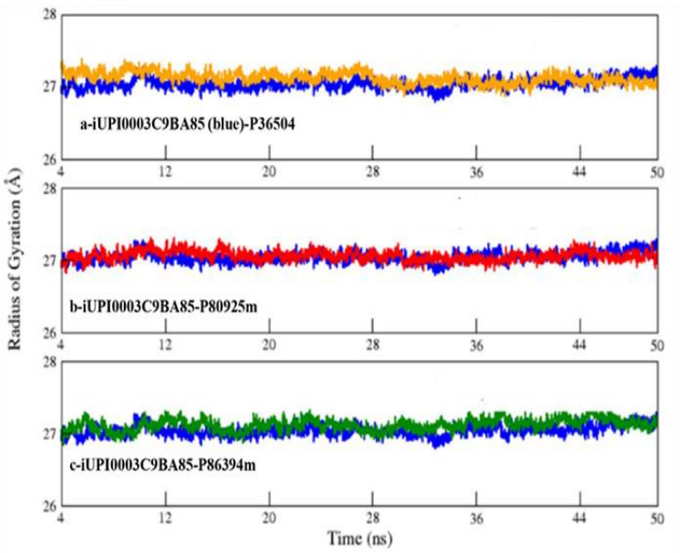

Figure 5: RoG Plot of UPI0003C9BA85 (blue) *(S. marcescens)* with its three peptides, a- UPI0003C9BA85, b- UPI0003C9BA85- P36504 complex, c-UPI0003C9BA85-P80925m complex, d-UPI0003C9BA85 -P86394m

Food spoiling agents include microbes (fungi and bacteria), insect pests, rodents, birds and physical damages (Rasch *et al.*, 2005). Microorganism contamination during storage and some pathogenic fungi species are mainly responsible for this kind of spoilage. In the current study, among the food spoilage bacteria, we have selected a species of *S. marcescens*. The Serratia is a genus containing gram negative pathogenic bacterial species. These are well adaptive in nature and facultative anaerobic in nature (Rawat, 2015). They are Serratia is a genus found in much diversified habitats including soil, fresh fruit and preserved food items (fruit, vegetable, juices meat and fish), plant and water (Grimont and Grimont, 2006). Most of the species, especially *S. liquefaciens* and *S. marcescens*, have been found to play role in fresh and preserved food items (Korcz *et al.*, 2021) But these are opportunist in nature and have a tendency to become human pathogenic when deprived of primary host (food items) and found conducive environment to infect human for their survival (Mahlen, 2011).

Preservatives play an essential role in many products used every day to help prevent the growth of harmful microorganisms and to protect products from contamination or spoilage (Smid and Gorris, 2007). Modern and industrial levels of preserving food, like canning and meat curing, typically involve three types of chemical preservatives: Benzoates (sodium benzoate) Nitrites (sodium nitrite) Sulphites (Sulphur dioxide)(Amit *et al.*, 2017). In addition, chemical food safety has emerged as a significant global issue with public health and international trade implications (Satcher, 2000). The use of chemicals in food preservation is very threatening in food industry in context of consumer health. Alternative and safer food preservation methods are emphasized to be used in current scenario (Johnson *et al.*, 2018). The use of chemical preservatives is discouraged by the scientists and health experts, bacteriocins, a natural food preservative, can help resolve these issues (Ng *et al.*, 2020b). Different nonchemical approaches have been introduced and encouraged in food preservation industry (Winkelströter et al., 2022). Among the suggested natural food storage agents, bacteriocins are preferred to be used as preservative agents in food

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storage either in fresh or processed food storage because of their proteolytic, variable range of pH and temperature tolerance (Kaya *et al.*, 2019). Scientists all over the world are showing their keen interest to isolate different types of bacteriocin producing strains and characterize bacteriocin produced by them for food preservation. These have been also reported to be used in infectious human and livestock infectious disease control (Ng *et al.*, 2020b). In the current study we have identified the effective bacteriocins to inhibit the functions of essential virulent proteins in *S. marcescens*. This study identified the bacteriocins that can target enzymes and proteins in food spoiling bacteria and played an essential role in the food preservation. Subtractive proteome approach has been popularize in current decade in the field of vaccinology and for identification of novel drug targets in the pathogenic microorganisms (bacteria and virus)(Hossain *et al.*, 2017).

To elucidate the impact of bacteriocin, we have retrieved amino acid sequences of 200 bacteriocins from UniProt database. Only72 peptides were selected for further study and 127 were discarded. Non immunogenic bacteriocins are considered as safe and effective bio preservatives (Lahiri *et al.*, 2022). So, only 56 non-immunogenic peptides were selected. A mutation is the replacement of an amino acid in the sequence, which might have structural effects on the resulting protein and hence have an effect on its function (Tóth-Petróczy and Tawfik, 2014). Point mutation was incorporated in 56 bacteriocin sequences by using I-Mutant 2.0. Resultantly we got 112 (56 wild type+ 56 mutant) peptides and 5 mutant peptides of non-antimicrobial wild type were found antimicrobial. In the stage II, proteome of *S. marcescens* was retrieved from the database and paralogs were eliminated from selected organism to avoid any redundant proteins, only non-paralogs were selected for further study using CD-HIT. Organisms depend on essential genes for development and reproduction.

The essential proteins in protein networks play an important role in complex cellular functions and in protein evolution. It is difficult to categorize genes as essential or non-essential because a gene's essentiality depends on a number of different circumstances (Zhang *et al.*, 2016). Numerous computational methods have been developed to determine important genes, and the majority of them train the mode using sequence-based information (Nigatu *et al.*, 2017). Total 2,711 bacterial essential protein sequences were downloaded from NetGenes for further analysis. It has predicted essential genes for bacterial organisms using network-based features. was used to identify the virulent proteins in our selected target organism because, A pathogen's ability to infect or damage its host tissues is determined by the virulence factors (Casadevall *et al.*, 2009). Total 112 Virulent proteins present in *S. marcescens* predicted by using VFDB (Virulence Factor Database). Subcellular localization critically influences protein function, and cells control protein localization to regulate biological processes (Yousefi *et al.*, 2021). An online web servers called PSORTb was used for identification of subcellular localization. Only 45 membranous proteins were selected for further study and the rest were discarded. Two proteins UPI0002AF278A and UPI0003C9BA85 were selected for protein-peptide docking, with bacteriocins. Three dimensional structures of pathogenic proteins and bacteriocin proteins were used for protein-peptide docking study. Protein-peptide docking techniques are currently most frequently used in experimental work supporting structure-based drug discovery and design, for instance to interpret ambiguous experimental data, identify important interactions, or simply for complicated visualization (Ciemny *et al.*, 2018). This technique is popularly used in computational vaccinology studies (Yousafi *et al.*, 2021). There are two steps in the computation for docking. The docking algorithm looks for the ligand's binding pose in the target protein's pocket in the first stage, and in the second step, binding affinity is assessed using a straightforward empirical scoring system. But empirical score are not a reliable predictor for correct and stable binding as it is calculated for one pose out of more other possible ones (Warren *et al.*, 2006). Other dynamics, like in cell environment, should be kept in consideration for calculating the empirical score (Yamashita *et al.*, 2014). Molecular dynamics simulations are set of computational methods to improve the methods of calculation of empirical scores (Yamashita *et al.*, 2015; Sadybekov and Katritch, 2023). It improves the calculation of free energy/empirical scores by providing the complex a diversified cell like environment (Fujitani *et al.*, 2005). Two protein-peptide complexes, UPI0003C9BA85-P36504, UPI0003C9BA85-P80925m and UPI0003C9BA85-P86394m, with good binding affinities and hydrogen bonding were selected for further MD simulation analysis ran through Amber packages (Jiang *et al.*, 2014). After completing MD simulations, to study the structural and conformational changes occurred in proteins and peptides, To examine protein behavior in a traditional MD simulation a time series of the root-mean-square deviation (RMSD) is calculated for Cα fluctuations (Carugo and Selection, 2007). In addition, an effective method for examining the structural stability of a protein is to plot different energies against RMSD (Harano *et al.*, 2007). It has been observed in current study that the RMSD fluctuation for 3peptides P36504 and P80925m and P86394m did not cause any large structural changes throughout simulations. All the peptides showed maximum stability throughout simulations of 50 ns complexed with UPI0003C9BA85 The root mean square fluctuation (RMSF) regarding the average position of residues defined by C-atoms is a commonly used metric to quantify changes in dynamics (Farmer *et al.*, 2017). Three complexes, UPI0003C9BA85-P36504, UPI0003C9BA85-P80925m and UPI0003C9BA85-P86394m showed almost same pattern with slight average value of RMSF fluctuation range from 1 to 2 Å. The compactness of protein and peptide structures was depicted by Radius of Gyration (Rg). If the Rg values remains steady during simulations it means protein or complex stability however, if unfolding occurs in protein the value does not remain same or fluctuates with respect to time during simulations (Lobanov *et al.*, 2008). Radius of gyration (ROG) were calculated and their plots were generated. All the complexes *i.e.*, UPI0003C9BA85-P36504, UPI0003C9BA85-P80925m and UPI0003C9BA85-P86394m showed maximum stability throughout simulations of 50 ns.

CONCLUSIONS: Bacteriocins were identified as food preservative after performing their molecular docking with virulent/ pathogenic proteins. Based on their complex stability during Molecular Dynamic simulation (UPI0003C9BA85), three bacteriocins (P36504, P80925m, P86394m) were selected for future research. The identified antimicrobial bacteriocins would be tested in wet lab for further confirmation. These predicted bacteriocins can be used as an alternate of chemical food preservative in food industry.

CONFLICT OF INTEREST: Authors have no conflict of interest

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