



In Silico Molecular Analysis and Docking of Potent Antimicrobial Peptides Against MurE Enzyme of Methicillin Resistant *Staphylococcus Aureus*

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Abstract

During the last decade there has been an alarming increase in the appearance of antibiotic-resistant bacteria. The drug-resistant microorganisms dubbed superbugs are projected to kill 10 million people a year by 2050. The annual frequency of deaths from Methicillin-resistant *Staphylococcus aureus* (MRSA) is rapidly increasing and surpassing those caused by human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS). Muramyl ligase E (MurE), an enzyme involved in the peptidoglycan biosynthesis of the bacterial cell wall, is a highly druggable target in MRSA. Six anti Methicillin-resistant *Staphylococcus Aureus* peptides were selected for deep bioinformatic analysis. The six anti-Methicillin-resistant *Staphylococcus Aureus* peptides were modeled and then docked to MurE ligase and their binding interactions were studied. The findings suggested that the interactions of three antimicrobial peptides (Flowlicidin-2, Ostricacin-2 and Ostricacin-3) with MurE could be essential for their inhibitory activity.

Keywords Methicillin-resistant *Staphylococcus Aureus* (MRSA) · AntiMRSA peptides · Molecular modeling · Molecular docking · Muramyl ligase E (MurE) · Antimicrobial peptides

Introduction

During the last decade there has been an alarming increase in the appearance of antibiotic-resistant bacteria as a result of an increased use of antibiotics combined with the

exceptional ability of bacteria to develop resistance (Singh and Mukhopadhyay 2014). The drug-resistant microorganisms dubbed superbugs are projected to kill 10 million people a year by 2050 and will overtake cancer as the leading cause of death worldwide (O'Neill 2016).

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are caused by strains of *Staphylococcus aureus* that have become resistant to the antibiotics (methicillin) commonly used to treat ordinary infections (Ryu et al. 2014). The annual frequency of deaths from MRSA is rapidly increasing and surpassing those caused by human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) (Bancroft 2007).

Antimicrobial peptides (AMPs) are produced in nature by plants, animals, bacteria and fungi and have been demonstrated to be quite effective in killing a wide selection of bacteria pathogens, including MRSA, and represent promising foundations for developing a new generation of antimicrobial agents (Wang 2010). Additionally, 60 therapeutic peptides have been approved by US Food and Drug Administration (FDA) as antibodies, vaccines, and antimicrobial agents, etc. (Aguilar-Toalá et al. 2019). It has been suggested for a long time that the main mechanism of action of AMPs

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targets the membrane (Boman 1995) and form pores by oligomerization. However, more recently, an activity on certain intracellular targets has been proven for peptides such as mersadecin (inhibiting the synthesis of peptidoglycan by interaction with the lipid H intermediate) (Brötz et al. 1998). Such activity could explain a divergence, for some peptides, between the speed of cell death and the speed of membrane destruction (Kim and Brogdeb 2005; Takahashi et al. 2010). Peptidoglycan is one of the main components of the bacterial cell wall, and it represents one of the most frequently used targets for antibacterial agents (Simcic et al. 2012). The mechanism of the peptidoglycan biosynthesis is one the best known and most validated drug targets for antibacterial therapy (Tomašić et al. 2012). The fact that Mur ligases are unique to prokaryotic cells and are vital for the survival of bacteria makes them promising emerging targets for novel antibacterial drug design (Kouidmi et al. 2014). These enzymes successively add L-Ala, D-Glu, meso-A2pm or L-Lys, and d-Ala-D-Ala to the nucleotide precursor UDP-MurNAc, (El Zoeiby et al. 2003; Barreteau et al. 2008). Knowing that compounds that interfere with the biosynthesis and assembly of peptidoglycan have been utilized as antimicrobial agents, Mur enzymes, which catalyze the cytoplasmic steps of the peptidoglycan biosynthesis, are advanced as excellent candidates for antibacterial drug discovery and development (Nikolaidis et al. 2014). Muramyl ligase E (MurE), an enzyme involved in the peptidoglycan biosynthesis of the bacterial cell wall, is a highly druggable target in MRSA (Billones and Bangalan 2019).

In silico analysis, involves computational methods applied to manage, curate, and interpret information related to biological systems (Li-Chan 2015). Bioinformatics approaches may overcome the high cost and the long time consuming by conventional methods, thereby enabling rapid research and study of bioactive peptide (Tu et al. 2018). In the present study, we have focused our attention on MurE which is a UDP-N-acetylmuramoylalanyl-D-glutamate-2, 6-diaminopimelate ligase, that adds m-DAP to UDP-MurNAc-L-Ala-D-Glu (Moraes et al. 2015). The Mur E enzyme of Mur pathway of MRSA is an attractive drug target as it is unique to bacteria and is absent in mammalian cells. MurE adds L-lysine to MRSA and other Gram-positive bacteria (Lto and Strominger 1964). It is therefore imperative to find efficient

peptides inhibitors of MurE enzyme, which is essential in the crucial step of prostaglandin biosynthesis (Billones and Bangalan 2019).

Molecular docking, as well as Quantitative structure-activity relationship also have been used to screen and predict the potential peptides (Nongonierma and Fitzgerald 2016). In the current study, six peptides were selected and subjected to further study involving homology modelling and molecular docking against MurE as enzyme target of MRSA strain.

Materials and Methods

Selected Anti-MRSA Peptides

From the 116 anti-MRSA collected peptides (Zouhir et al. 2016), six antiMRSA peptides were selected for further bioinformatic analysis namely: Flowlicidin-2, Pilosulin-1, Pleurocidin, Ostricacin-1, Ostricacin-2 and Ostricacin-3. The choice of these peptides is based on antiMRSA activity and their short sequence (Table 1).

Sequences Analysis

CAMPSign tool (<http://www.campsign.bicnirrh.res.in>) was used for identification of AMPs belonging to one of the 45 major AMP families present in CAMP_{R3} (Waghu et al. 2016). The peptides are identified based on family-specific sequence signatures such as patterns and Hidden Markov Models (HMMs) generated for experimentally known AMPs.

ProtParam online tool (<http://web.expasy.org/protparam/>) was used to determine the physico-chemical parameters of the six selected antiMRSA peptides.

The prediction of peptide antimicrobial activity was performed using the most recent version of: antimicrobial peptide database (APD3) (Wang et al. 2016) and using “Yet Another Database” of AntiMicrobial Peptides (YADAMP) (<http://yadamp.unisa.it/>) (Stefano et al. 2012).

Table 1 The primary structures of the six selected antiMRSA peptides

Peptide anti-MRSA name	Sequence
Pilosulin-1	GLGSVFGRLARILGRVIPKV
Pleurocidin	GWGSFFKKAHVKGKGVGKAALTHYL
Flowlicidin-2	LVQRGRFGRFLRKIRRFPRPKVTITIQGSARFG
Ostricacin-1	LFRCRKGTCFVGGCPAHLVKVSGSCFGFRACCKWPWDV
Ostricacin-3	IPRPLDPCIAQNGRCFTGICRYPYFWIGTCRNGKSCRRR
Ostricacin-2	APGNKAECEREKGYCGFLKCSFPFVVSGBKCSRFVFCCKNIW

Molecular Modeling and Models Validation

The MurE enzyme sequence used for homology is that of MRSA 252, was retrieved from UniProt Data Bank (<https://www.uniprot.org/>). The automated protein structure homology modeling server, Swiss Model (<http://www.expasy.org/swissmod/>), was used to generate the three-dimensional structural model of MurE enzyme using the crystal structure of MuE enzyme from *Staphylococcus aureus* MurE (PDB code 4C12) as a template since both enzymes shared about 98.99 % of identity and 100 % of sequence coverage.

Swiss model server was also used to generate the 3D structural model of 4 peptides having a sequence longer than 30 amino acids. For the two short peptides Pleurocidin and Pilosulin 1, PEPstrMOD method (Singh et al. 2015) was used for structure prediction. Peptides models were further refined by the interactive web server 3Drefine publicly available at the URL: <http://sysbio.rnet.missouri.edu/3Drefine/> (Bhattacharya et al. 2016). Structures were therefore evaluated and validated by using SAVES v5.0 (Structural Analysis and VERification Server) was used for further checking and validating protein or peptides structures. SAVES (<https://services.mbi.ucla.edu/SAVES/>) utilizes 6 programs for doing this: Verify3D, ERRAT, Prove, PROCHECK, WHATCHECK and CRYST. Verify3D (Luthy et al. 1992; Bowie et al. 1991) was used to verify the packing quality of each residue in the model. PROCHECK was used to determine the torsion angle restraints for side chains of each amino acid in the predicted models (Laskowski et al. 1993) and thereby to draw Ramachandran plots.

The PyMOL v0.99 program (<http://www.pymol.org>) was used to visualize and analyze the generated model structure and to construct graphical presentations and illustrative figures.

Protein–Protein Docking Analysis

Protein-protein docking of the antiMRSA peptides with the MurE enzyme was performed using the HDock server (<http://hdock.phys.hust.edu.cn/>). The server automatically predicts the interaction between receptor and ligand molecules through a hybrid algorithm of template-based modeling and ab initio template-free docking. HDock incorporates the binding interface information from the PDB and/or user-input biological information like residue restraints and molecular size/shape information obtained from small-angle X-ray scattering, supports both amino acid sequence and structure inputs and uses an intrinsic

scoring function for protein-protein interactions. During docking calculation, the top 10 orientations with the best shape complementarity scores in 3D translational space are evaluated by a scoring function (ITScorePP) and the top-scored one is kept (Yan et al. 2020).

Interface covalent bonds, hydrogen bonds and salt bridges between antiMRSA peptide and MurE enzyme within the predicted docking models were evaluated using the online tool PDBePISA (<http://www.ebi.ac.uk/pdbe/pisa/>).

The PyMOL program was ultimately used for the visualization of structures showing the interactions between toxin and receptor.

Results and Discussion

Analysis of Selected AntiMRSA Peptides

Through CAMP_{R3} database, the three Ostricacin peptides were identified as Defensin family members, Flowlicidin-2 as Cathelicidin family member, Pleurocidin as Pleurocidin member while Pilosulin-1 was not identified to belong to any AMP family.

The physico-chemical analysis of the six selected anti-MRSA was performed using ProtParam tool (Table 2). The inspection showed that the number of amino acids vary from 20 to 41 residues with a molecular weight ranging from 2109 to 4689 Da. All the pHi are in the basic zone and the highest value (12.85) is given by Flowlicidin-2. All peptides have high estimated half-life expect Flowlicidin-2 and Ostricacin-1 with exhibit the lowest estimated half-life when expressed in Yeast (3 min) or *E. coli* (2 min). Among the six peptides, only Ostricacin-2 was predicted to be unstable.

The antimicrobial peptide database (APD3) (Wang et al. 2016) and yet another database of antimicrobial peptides (YADAMP) (<http://yadamp.unisa.it/>) (Stefano et al. 2012) databases were used for prediction of peptide antimicrobial activity. As shown by Table 3, Pleurocidin, Ostricacin-1, Ostricacin-2 and Ostricacin-3 exhibit antiMRSA activity and have different animal sources. However, Flowlicidin-2 and Pilosulin-1 have not been recognized by this database.

Homology Modeling and Structural Inspection

To gain further insights into the structure of the 6 selected peptides, a search for homology modeling. According to the peptide length, Swiss-model server was used to predict the models of Flowlicidin-2, Ostricacin-1, Ostricacin-2 and Ostricacin-3 based on available RMN structures, while models of Pleurocidin and Pilosulin-1 were predicted using PEPstrMOD method (Singh et al. 2015) recommended for short

peptides. Related Data to modeling method, PDB template, model oligomerization, sequence identity between peptide and template as well as sequence coverage are summarized in Table 4.

To validate the predicted models, their stereochemical quality was assessed by Ramachandran plots (Fig. 1). All models showed a high percent of residues (ranging from 95

to 100%) in the most favored regions and allowed regions. A very low percent of residues in the disallowed region supports high geometry of models. In addition, the packing quality of each residue in the model was evaluated using Verify 3D. For all models, the majority of residues have a score indicating acceptable side chain environment and reasonably good structures. Accordingly, and based on all

Table 2 Physico-chemical analysis of the six selected antiMRSA peptides as determined by ProtParam Tool

Peptide name	Number of amino acids	Molecular weight (Da)	pHi	Estimated half-life	Instability index
Pleurocidin	25	2711.17	10.18	30 h (in mammalian reticulocytes) > 20 h (in yeast) > 10 h (in <i>Escherichia coli</i>)	= 4.97 This classifies the protein as stable
Pilosulin-1	20	2108.60	12.30	30 h (in mammalian reticulocytes) > 20 h (in yeast) > 10 h (in <i>Escherichia coli</i>)	= 13.44 This classifies the protein as stable
Flowlicidin-2	32	3818.58	12.85	5.5 h (in mammalian reticulocytes) 3 min (in yeast) 2 min (in <i>Escherichia coli</i>)	= 29.01 This classifies the protein as stable
Ostricacin-1	36	4017.80	8.94	5.5 h (in mammalian reticulocytes) 3 min (in yeast) 2 min (in <i>Escherichia coli</i>)	= 37.89 This classifies the protein as stable.
Ostricacin-2	41	4688.51	8.89	4.4 h (in mammalian reticulocytes) > 20 h (in yeast) > 10 h (in <i>Escherichia coli</i>)	45.40 This classifies the protein as unstable
Ostricacin-3	40	4679.52	9.69	20 h (in mammalian reticulocytes) 30 min (in yeast) > 10 h (in <i>Escherichia coli</i>)	= 35.15 This classifies the protein as stable

Table 3 Antimicrobial activity prediction of the studied antiMRSA peptides via the (APD3) and (YADAMP)

Peptide name	APD Number/ YADAMP ID	Source	Activity
Flowlicidin-2	Not found	<i>Gallus gallus</i> (Chicken) (Zouhir et al. 2016)	Anti-MRSA
Pilosulin-1	ID 946	<i>Mymecia pilosula</i> (Ant)	Anti-Gram+ & Gram-, AntiMRSA
Pleurocidin	AP00166	Skin mucous secretions, Winter flounder, <i>Pleuronectes americanus</i>	Anti-Gram+ & Gram-, Antifungal, candidacidal, Chemotactic, Antibiofilm
Ostricacin-1	AP01320	<i>Struthio camelus</i>	Anti-Gram+ & Gram-, AntiMRSA, anti-sepsis
Ostricacin-2	AP01321	<i>Struthio camelus</i>	Anti-Gram+ & Gram-, Antifungal, candidacidal, AntiMRSA, antisepsis
Ostricacin-3	AP01322	<i>Struthio camelus</i>	Anti-Gram+ & Gram-, AntiMRSA

Table 4 Data about homology modeling of selected antiMRSA peptides

Peptide	Modeling server	PDB template	PDB description	Identity (%)	Coverage (range)	Model Oligo state
Pleurocidin	PEPstrMOD	–	–	–	–	Monomer
Pilosulin-1	PEPstrMOD	–	–	–	–	Monomer
Flowlicidin-2	Swiss Model	2gdl	Myeloid antimicrobial peptide 27	100 %	0.97 (1–31)	Monomer
Ostricacin-1	Swiss Model	2lg6	Gallinacin-2	75 %	1 (1–36)	Monomer
Ostricacin-2	Swiss Model	1ut3	Spheniscin-2	37.84 %	0.90 (5–41)	Monomer
Ostricacin-3	Swiss Model	5lcs	Gallinacin-7	69.23 %	0.98 (1–39)	Monomer

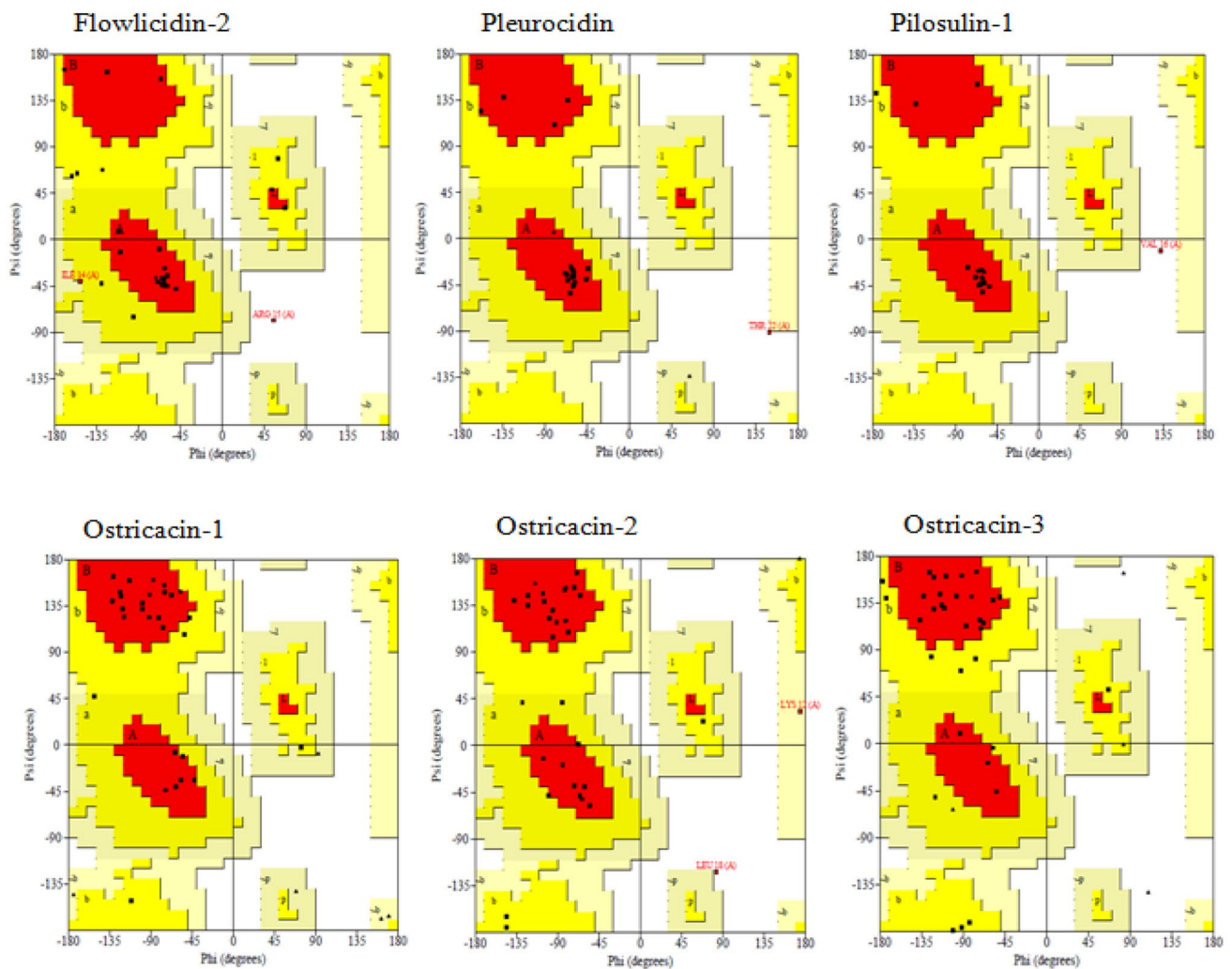


Fig. 1 Ramachandran plots of six modeled antiMRSA peptides

obtained data, predicted models were validated and retained for further investigation.

The 3D structure inspection revealed huge structural divergence between the 6 selected antiMRSA peptides (Fig. 2). For Ostricacin peptides, the tertiary structure is principally marked by the occurrence of anti-parallel β -strands. However, Flowlicidin-2 is especially marked by a helical organization at the C-terminal extremity. Pilosulin-1 and Pleurocidin models indicated the presence of a noticeable helix at the N-terminal end.

Structure Inspection of MurE Enzyme

MurE enzyme of MRSA 252 is a 494 amino acids protein with about 54.153 kDa and a theoretical pHi of 5.32 as revealed by ProtParam analysis. The 3D-structural model of MurE was predicted using the crystal structure

of MuE enzyme from *Staphylococcus aureus* MurE (PDB code 4C12) (Ruane et al. 2013) as a template since both enzymes shared about 98.99% of identity and 100% of sequence coverage. The Ramachandran plot analysis of the generated model revealed that 960 residues (98%) of the model were in the favored, 16 residues (1.6%) in the allowed regions and only 4 residues (0.4%) in the disallowed regions. As more than 99% of the residues were in the favored and allowed regions, the model was consequently validated. The model was also checked and validated using the structural analysis and verification server (SAVES) and accordingly it was retained for analysis and further investigation.

The examination of the MurE enzyme model showed a homodimer organization (Fig. 3) and the presence of 8 ligands (4 Mg^{2+} ions, 2 UML and 2 UDP) establishing several interactions with the protein residues as illustrated in Table 5.

Fig. 2 Ribbon representation of the predicted 3D structures of six antiMRSA peptides. The N-terminal end is indicated in blue and the C-terminal end in red

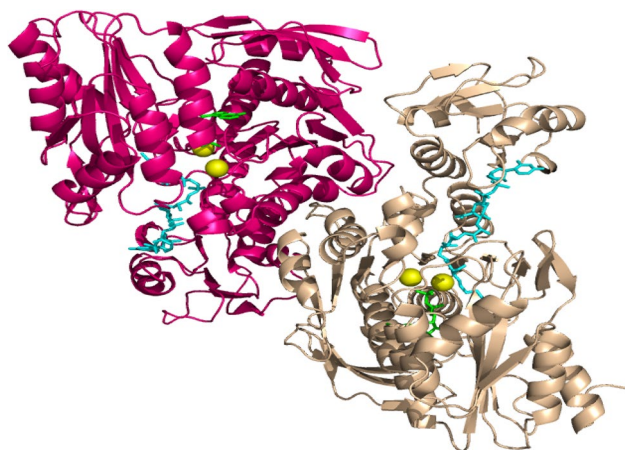
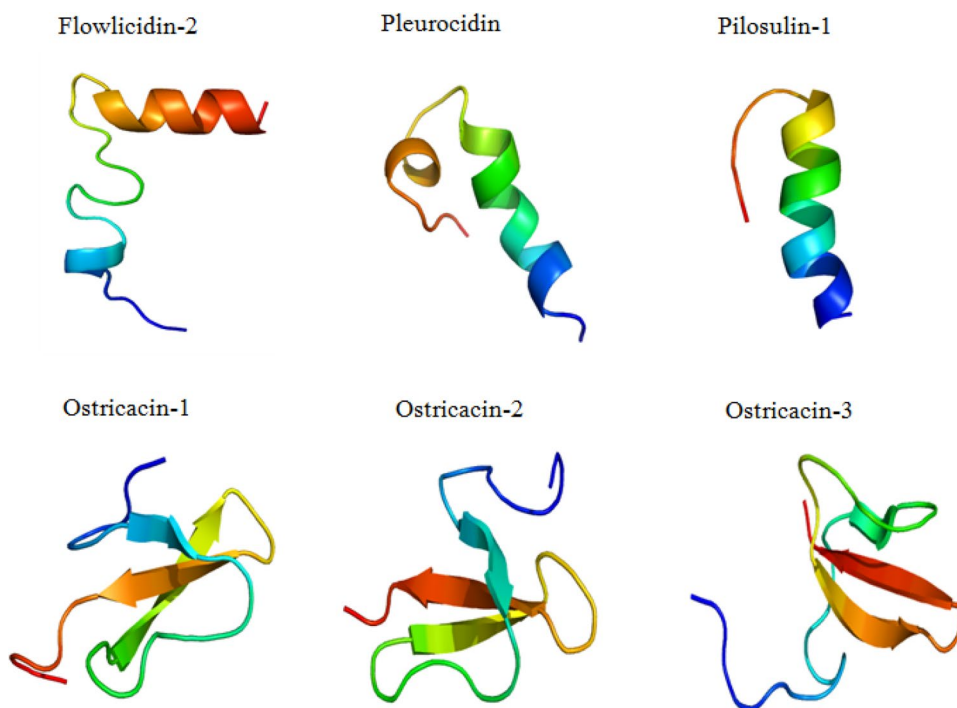


Fig. 3 Overview of MurE enzyme 3D structure in ribbon representation. Chain A: pink; Chain B: wheat; UML3 and UML9: cyan sticks; 2 ADP: green sticks; Mg ions: yellow spheres (Color figure online)

MurE-antiMRSA Docking Analysis

The protein-protein docking models of antiMRSA peptides and MurE enzyme were predicted by the updated version of HDOCK server (Yan et al. 2020). As shown by Fig. 4, the binding of antiMRSA peptides to MurE enzyme is occurred in different manner. Subsequently, the interface interactions between the enzyme and the coupled peptide were evaluated using PDBePISA to more elucidate the binding mode and involved interactions and

residues (Table 6). Interestingly, among the six peptides, only Flowlicidin-2 established interactions with UML9 via covalent bonds. These covalent bonds could be responsible of irreversible inhibition which could explain the low minimum inhibitory concentration (MIC) and then the high observed antimicrobial activity of Flowlicidin-2 (Table 7).

On the other hand, Ostricacin-2 exhibited interactions with only two amino acid in the chain B namely Glu246 and Glu264 (Fig. 5) and has an antimicrobial activity very closed to Flowlicidin-2 (1.59 versus 1.58 $\mu\text{g}/\text{ml}$). In addition, Pilosulin-1, Pleurocidin, Ostricacin-1 and Ostricacin-3 interacted with both chain A and B of MurE enzyme. Ostricacin-3 which had a good antimicrobial activity (Table 7) exhibited the highest number of interactions between peptide-MurE when compared with other studied AMPs. In order to verify whether the number or the type (covalent or uncovalent) of interactions peptide-MurE is essential for the efficiency of the anti-microbial peptides, The peptides should be generated, tested in the same conditions and even site-directed mutated to alter binding mode and confirm hypothesis.

Conclusions

Analysis of the bioactive peptides by conventional methods is expensive and time-consuming. Emerging bioinformatics approaches may overcome these drawbacks to enable rapid research and study of bioactive peptide (Tu et al. 2018). In the current work, diverse methods *in silico*

Table 5 Data about the MurE enzyme three-dimensional model (oligomerization state, template and interactions established with ligands)

Oligo State	Template	Ligand	Interactions
Homodimer	4C12 98,99 % Identity 100 % Coverage	4 magnesium ions: Mg5 Mg6 Mg11 Mg12 2 URIDINE 5'DIPHOSPHO N-ACETYL MURAMOYL-L-ALANYL-D-GLUTAMYL-L-LYSINE (UML): UML3 UML9 2 ADENOSINE-5'-DIPHOSPHATE (ADP) : ADP4 ADP10	Chain A: T115, T137, T152, E177, Ligand: ADP4 Chain A: G110, T111, K114, D204, H205, Y215 Chain B: T115, T137, T152, E177 Ligand: ADP10 Chain B: G110, T111, K114, D204, H205, Y215 Chain A: T28, D29, S30, R31, F39, S42, G44, Y45, T46, V47, S49, F52, A150, N151, T152, T153, E155, S179, H181, R187, H205, Y351, H353, R383, D406, N407, S456, E460 Chain B: T28, D29, S30, R31, F39, S42, G44, Y45, T46, V47, S49, F52, A150, N151, T152, T153, E155, S179, H181, R187, H205, Y351, H353, R383, D406, N407, S456, E460 Chain A: T111, N112, G113, K114, T115, S116, E177, N200, F300, N304, R335, D350, Y351, A352, H353, D356, G357, K360, L361 Chain B: T111, N112, G113, K114, T115, S116, E177, N200, F300, N304, R335, D350, Y351, A352, H353, D356, G357, K360, L361

were applied in order to analyze the properties and the structure of antiMRSA peptides as well as their interactions with MurE enzyme. The six anti-MRSA peptides were then docked to the enzyme target and their binding energies were calculated. Three AMPs (Flowlicidin-2, Ostricacin-2 and Ostricacin-3) displayed greater inhibitory potential against MurE ligase compared to the others. These three peptides can be chemically synthesized and their activities against the MRSA strain can be tested in further study.

This study is an essential step to understand the mode of action of antiMRSA peptides on Mur enzymes such as MurE of MRSA.

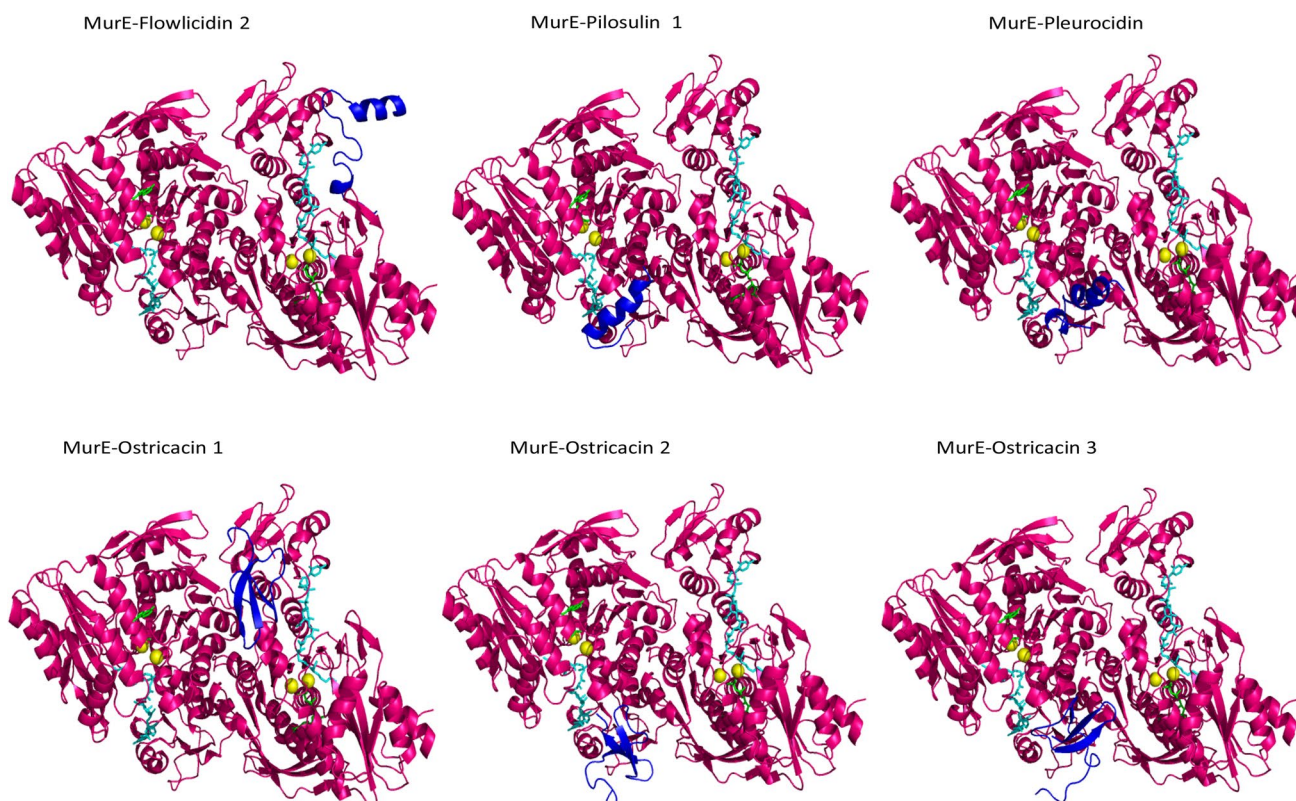


Fig. 4 Docking models of MurE enzyme (pink) with the six antiMRSA peptides (blue). Proteins are illustrated by cartoon representation; Mg ions by spheres; UMP and ADP by sticks (Color figure online)

Fig. 5 Overview of interactions between MurE and Flowlicidin-2, Ostricacin-2, Ostricacin-3. MurE enzyme (pink), antiMRSA peptides (blue), Hydrogen bonds and salt bridges (black dishes), involved amino acids are represented by sticks

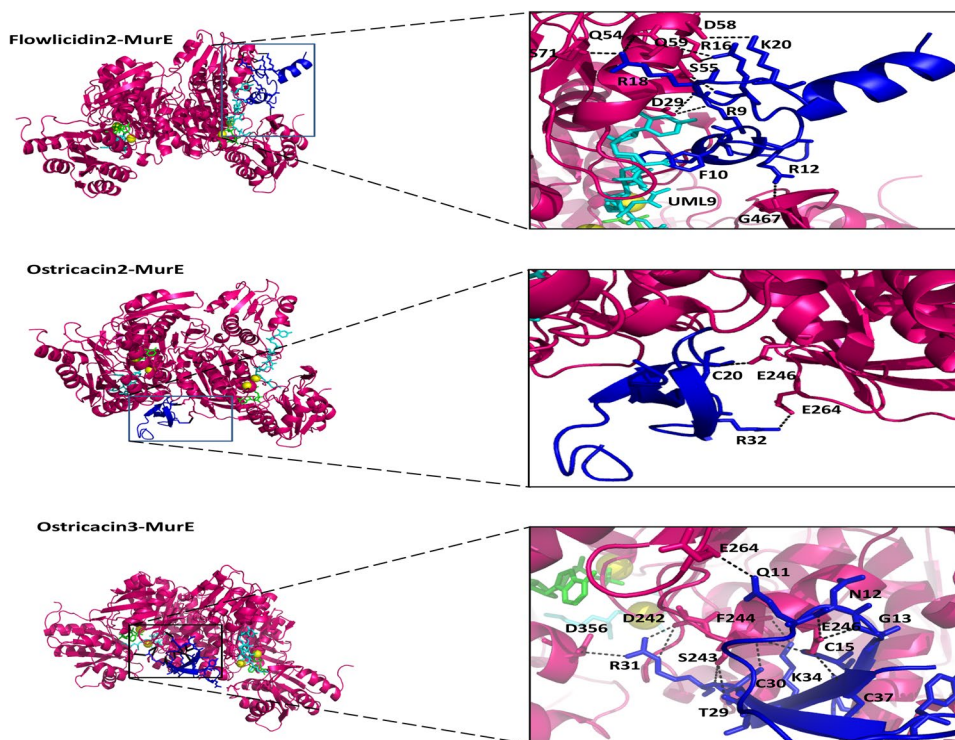


Table 6 Comparative analysis of binding interactions between MurE enzyme and modeled antiMRSA peptides

Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Flowlicidin-2
Hydrogen bonds	1	Ser55 [OG]	3.00	Arg16 [O]
	2	Asp29 [OD2]	3.75	Arg9 [NE]
	3	Asp29 [OD2]	3.78	Arg9 [NH2]
	4	Gly467 [O]	2.88	Arg12 [NH1]
	5	Gln59 [OE1]	2.77	Arg16 [NH1]
	6	Ser55 [O]	3.11	Arg16 [NH1]
	7	Gln54 [OE1]	3.62	Arg18 [NH2]
	8	Ser71 [O]	2.72	Arg18 [NH2]
Salt Bridges	1	Asp29 [OD2]	3.75	Arg9 [NE]
	2	Asp29 [OD2]	3.78	Arg9 [NH2]
	3	Asp58 [OD2]	3.69	Lys20 [NZ]
Interactions	No.	MurE-enzyme UML9	Distance (Å)	AMP Flowlicidin-2
Covalent bonds	1	O2A	1.48	Phe10 [CE1]
	2	O2A	1.24	Phe10 [CZ]
Hydrogen bond	1	O6'	2.66	Arg6 [NH2]
Interactions	No.	MurE-enzyme Chain A	Distance (Å)	AMP Pilosulin-1
Hydrogen bonds	1	His209 [ND1]	2.60	Ser4 [OG]
	2	Val43 [O]	3.79	Arg8 [NH2]
	3	Tyr45 [OH]	3.12	Arg11 [NE]
	4	His209 [O]	3.28	Ser4 [N]
Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Pilosulin-1
Hydrogen bond	1	Tyr247 [OH]	3.19	Gly1 [N]
Interactions	No.	MurE-enzyme Chain A	Distance (Å)	AMP Pleurocidin
Hydrogen bonds	1	Tyr45 [OH]	3.13	Lys14 [O]
	2	Thr211 [OG1]	3.55	Lys8 [NZ]
	3	Leu185 [O]	3.6	His15 [ND1]
Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Pleurocidin
Hydrogen bonds	1	Glu213 [OE1]	2.33	Trp2 [NE1]
	2	Glu213 [OE1]	3.10	Phe5 [N]
	3	Glu213 [OE2]	2.26	Lys8 [NZ]
Salt bridges	1	Glu213 [OE1]	3.71	Lys8 [NZ]
	2	Glu213 [OE2]	2.26	Lys8 [NZ]
Interactions	No.	MurE-enzyme Chain A	Distance (Å)	AMP Ostricacin-1
Hydrogen bonds	1	Asn240 [OD1]	3.24	Arg4 [NE]
	2	Asn240 [O]	2.60	Arg4 [NH1]
	3	Asp242 [O]	2.41	Arg4 [NH2]
Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Ostricacin-1
Hydrogen bond	1	Val43 [O]	3.23	Thr7 [OG1]

Table 6 (continued)

Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Ostricacin-1
Salt bridge	1	Asp48 [OD2]	3.49	His16 [ND1]
Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Ostricacin-2
Hydrogen bonds	1	Glu246 [OE2]	2.92	Cys20 [N]
	2	Glu264 [OE2]	2.77	Arg32 [NH2]
Salt bridge	1	Glu264 [OE2]	2.77	Arg32 [NH2]
Interactions	No.	MurE-enzyme Chain A	Distance (Å)	AMP Ostricacin-3
Hydrogen bonds	1	Thr211 [OG1]	2.87	Thr17 [O]
	2	Tyr45 [OH]	2.50	Tyr24 [OH]
Salt bridge	1	Asp384	3.44	Arg21 [NH1]
Interactions	No.	MurE-enzyme Chain B	Distance (Å)	AMP Ostricacin-3
Hydrogen bonds	1	Ser243 [OG]	3.56	Thr29 [O]
	2	Glu264 [O]	3.38	Gln11 [NE2]
	3	Glu246 [OE2]	2.95	Asn12 [N]
	4	Glu246 [OE2]	3.04	Gly13 [N]
	5	Ser243 [O]	3.84	Cys15 [SG]
	6	Glu246 [OE1]	2.03	Cys15 [SG]
	7	Ser243 [O]	3.09	Cys30 [SG]
	8	Ser243 [OG]	3.77	Arg31 [N]
	9	Asp242 [OD2]	2.82	Arg31 [NH2]
	10	Phe244 [O]	3.54	Lys34 [NZ]
	11	Glu246 [OE1]	3.74	Cys37 [N]
Salt bridges	1	Asp242 [OD2]	3.84	Arg31 [NE]
	2	Asp356 [OD1]	3.97	Arg31 [NH1]
	3	Asp242 [OD2]	2.82	Arg31 [NH2]

Table 7 Minimum inhibitory concentration (MIC) values of selected antiMRSA peptides as reported by Zouhir et al. (2016)

AntiMRSA peptides	Flowlicidin-2	Pilosulin-1	Pleurocidin	Ostricacin-1	Ostricacin-2	Ostricacin-3
MIC (µg/ml)	1.58	6.75	10.98	1.66	1.59	3.54

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed Consent All authors gave informed consent to the submission of this manuscript.

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