

## Modelling and Computational Sequence Analysis of a Bacteriocin Isolated from *Bacillus licheniformis* strain BTHT8

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### ABSTRACT

Bacteriocins are ribosomally synthesized antimicrobial peptides displaying different mode of action. Non pathogenic *Bacillus licheniformis* are usually associated with plants and produce several bacteriocins. This work looks at drug designing strategies using protein modelling and bioinformatics tools. In this study computational modelling and docking studies were carried out using the 13 amino acid sequence of a previously characterized thermostable bacteriocin, BL8 produced by *B. licheniformis* strain BTHT8. These analyses enabled the understanding of the structure of BL8 protein domain, which was noted to belong to alpha+beta protein fold family. In addition, the docking study predicted that BL8 has properties of angiotensin-converting enzyme (ACE) inhibitor.

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## 1. INTRODUCTION

Bacteriocins produced by microorganisms belonging to different eubacterial taxonomic branches exhibit bactericidal activity against closely related bacteria. Being heterogeneous compounds, they display variable molecular weights, biochemical properties, inhibitory spectra and mechanisms of action [1].

*Bacillus licheniformis* are non-pathogenic soil organisms mainly associated with plant and plant materials in nature, but can be isolated from nearly everywhere because of the dissemination of their highly resistant endospores with dust [2]. They also produce several bacteriocins [3, 4, 5].

Numerous online servers and tools predict 3D structure of proteins, while some others provide precise information on proteins interactions and their localization in stable conformation [6]. Modelling is the most common protein structure prediction methods in structural genomics and proteomics [7]. Computational biology and bioinformatics have the potential of speeding up drug discovery process thus reducing costs, and also changing the way drugs are designed [8]. One such method is the docking of the drug molecule with the receptor (target). The site of drug action ultimately responsible for the pharmaceutical effect is important in molecular modelling. Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation may therefore be used to predict the strength of association or binding affinity between two molecules. Docking predicts the binding orientation of small molecule- drug candidates to their protein targets, in order to predict the affinity and activity of the same

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[9]. The protein sequence available in the BIOPEP database can thus be evaluated as a good/bad precursor of peptides with biological activity as activity depends on amino acid composition and structure.

Angiotensin-I-converting enzyme (EC 3.4.15.1; ACE) was originally isolated from horse blood in 1956 as a hypertension-converting enzyme and plays a crucial role in regulating blood pressure by not only converting angiotensin-I to the potent vasoconstrictor angiotensin-II, but also by inactivating the vasodilator, bradykinin. Angiotensin-II causes the release of a sodium retaining steroid, aldosterone from the adrenal cortex, which increases blood pressure. Inhibition of ACE is a useful therapeutic approach in the treatment of hypertension. Search for natural ACE inhibitors as alternatives to synthetic ones is of great interest for safe and economical use as pharmaceuticals. Naturally occurring peptides with ACE- inhibitory activity were first obtained from snake venom. Oshima et al. [10] reported the first ACE inhibitory peptides produced from food proteins by digestive proteases. Many marine-derived antihypertensive peptides had potent ACE inhibition activities [11]. These inhibitors bind to the active site or to the inhibitor binding site that is remote from the active site to alter enzyme conformation such that, the substrate no longer binds to the active site. Inhibitors of ACE are a first line of therapy for hypertension, heart failure, myocardial infarction and diabetic nephropathy.

Thus, here we tried to find the binding affinity of peptide BL8 which could go on to be a lead molecule. BL8 is a novel previously characterized 13 amino acid, thermostable peptide bacteriocin from *Bacillus licheniformis* BTHT8 isolated from marine sediment [2]. The aim of the present study was to perform computational sequence analysis and 3D structural modelling using this 13 amino acid sequence of BL8. A computational approach could be helpful in finding some structural requirements for peptides, to be designated as biologically active (bioactive) [12].

## 2. RESEARCH METHOD

BL8 sequence: NH<sub>2</sub>-Ser-Trp-Ser-Cys-Cys-Gly-Asn-Cys-Ser-Ile-Ser-Gly-Ser-COOH<sup>2</sup> was used in the analysis.

### 2.1 Protein sequence and structure analysis

Various bonding interactions between the side chains on the amino acid residues are used to determine the tertiary structure of the protein. I-TASSER server was used for modelling. I-TASSER simulations generate a large ensemble of structural conformations for each target called as decoys. For the selection of final model, I-TASSER [13], used the SPICKER program to cluster all the decoys based on pairwise structure similarity, reporting five models corresponding to the five largest structure clusters [14]. The confidence of each model is quantitatively measured by C-score that can be calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations.

### 2.1 Screening of bacteriocin BL8 for biological activity

A database of potential biologically active peptide sequences, named BIOPEP (<http://www.uwm.edu.pl/biochemia>) was used to screen the protein BL8 for its potential biological activity.

### 2.3 Preparation for Docking

Based on leads obtained from BIOPEP, the crystal structure of human angiotensin converting enzyme (PDB ID: 1O86) was downloaded from the database Protein Data Bank (PDB) (<http://www.rcsb.org/pdb/home/home.do>)<sup>15</sup>.

#### 2.3.1 Models predicted by I-TASSER

The model of the bacteriocin BL8 with the least C-score provided by I-TASSER server was used as the ligand for docking.

#### 2.3.2 Docking and visualization

For docking, we used 1O8A (PDB), a crystal structure of human ACE bound to lisinopril (ACE-inhibitory drug). A binding site with a radius of 15 Å and coordinates x: 41.268, y: 34.559 and z: 45.393 was created by the removal of lisinopril from ACE structure. Automated molecular docking was performed using the ZDOCK. ZDOCK is a rigid-body protein-protein docking algorithm based on the Fast Fourier Transform correlation technique that is used to explore the rotational and translational space of a protein-protein system [16]. Evaluation of the molecular docking was performed according to the scores and binding energy values in order to obtain the best peptide poses. All obtained conformations of protein - ligand complexes were analyzed for the interactions (the hydrophobic, hydrophilic, electrostatic, and coordination interactions) and binding energy of the docked structure using BIOVIA Discovery Studio Visualizer version 16.1.0.

## 3. RESULTS AND ANALYSIS

*Bacillus licheniformis* BTHT8 produced the thermostable bacteriocin BL8 whose sequence was revealed using N-terminal sequencing. This small peptides' 13 amino acid sequence stretch is: NH<sub>2</sub>-Ser-Trp-Ser-Cys-Cys-Gly-Asn-Cys-Ser-Ile-Ser-Gly-Ser-COOH [2] (SWSCCGNCSISGS). The novelty of this small peptide BL8 was disclosed when it was proved that the N-terminal amino acid sequences of BL8 showed no significant similarity with the sequences of bacteriocins of other *Bacillus* sp. The sequences also did not show

similarity when compared with bacteriocins from other organisms by BLAST in protein database of NCBI<sup>2</sup>. Therefore considering the novelty of the sequences, it was used for modelling and docking studies, to understand whether this peptide has other biochemical properties.

### 3.1. Modelling

The 13 amino acid sequence was modelled to predict its structure (Fig. 1). For each target, I-TASSER simulations generated a large ensemble of structural conformations called decoys. To select the final models, I-TASSER used the SPICKER program to cluster all the decoys based on the pair-wise structure similarity, and reported up to five models corresponding to the five largest structure clusters. C-score was typically in the range of [-5, 2], where a C-score of a higher value signifies a model with a higher confidence and vice-versa [17].

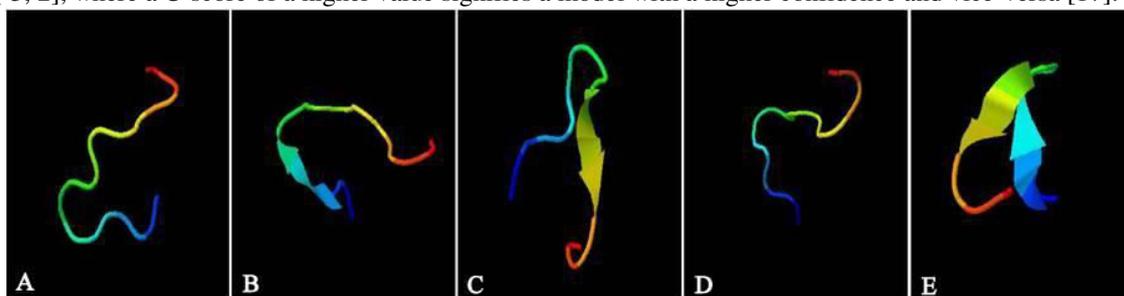


Fig. 1. Models of BL8 as predicted by I-TASSER : A) Model 1, B) Model 2 , C) Model 3, D) Model 4, E) Model 5 ; with C-score values as follows : -0.94, -1.22, -2.35, -2.64, -1.31 respectively. Model A with the highest C-score was considered as the best model.

### 3.2. Screening of protein BL8 for biological activity

BIOPEP revealed the potential biological activity of BL8. The frequency of the occurrence of fragments with the activity for the analyzed sequence is shown in figure 2. The analyzed peptide revealed the potential inhibitory activity on angiotensin-converting enzyme (ACE), i.e. its biological activity as an Angiotensin-converting enzyme inhibitor (ACE inhibitor). Through the use of BIOPEP server, the protein sequence of BL8 could be evaluated as a good/bad precursor of peptides with its biological activity showing it to be ACE inhibitor [18].

BIOPEP: Profiles of proteins potential biological activity					
Protein sequence:SWSCCGNCSISGS					
ID	Name of peptide	Activity	Number	Sequence	Location
7607	ACE inhibitor	ACE inhibitor	1	GS	[12-13]
7618	ACE inhibitor	ACE inhibitor	1	SG	[11-12]

Fig. 2. Frequency of occurrence of ACE inhibitory activity in BL8 sequence.

### 3.3 Docking studies

Contributors have to pay the following additional cost Since the biological function of the 13 amino acid peptide BL8 was shown to be Angiotensin-converting enzyme inhibitor (ACE inhibitor), it was necessary to understand the interaction of this peptide with ACE. Hence, in this study the docking interaction was performed between the receptor ACE (PDB ID 1O86) and ligand BL8 protein, using ZDOCK version, 3.0.2. [16]. This helped us to understand and explore their binding modes.

Knowledge of Angiotensin-converting enzyme (ACE) structure is essential for understanding as well as elucidation of the mechanism of action underlying the pharmacological and toxicological action of these agents for rational drug design. Somatic angiotensin-converting enzyme (ACE) contains two homologous domains (ACE<sub>N</sub> and ACE<sub>C</sub>), each bearing a functional active site [19] ACE possesses a catalytic zinc site which usually coordinates with nitrogen, oxygen and sulphur donors of His, Glu, Asp and Cys residues while His is most frequently encountered in the coordination sphere of zinc metal ion [20]. 36-residue synthetic peptides represent the ACE<sub>N</sub> [His361-Ala396] and ACE<sub>C</sub> [His959-Ala994] zinc binding sequences.

ZDOCK predicted several protein (ACE) – ligand (BL8) complexes of which, complexes I showed maximum favoured interactions. In complex I, the ligand interacting atoms of ACE comprised those of amino acid residues such as: Thr 92, Glu 403, Phe 512, His 513, Val 518, Tyr 135, Asn 136, Arg 124, Lys 118, Ile 88, Tyr 62, Trp 59, Tyr 360, Ala 63, Arg 402, Asn 66, Trp 357, His 353, Tyr 523, His 410, His 387, Glu 384, Ser 512 (Fig. 3). As most of the amino acid residues in the active site are hydrophobic, they were the major contributors to the receptor-ligand interaction [3].

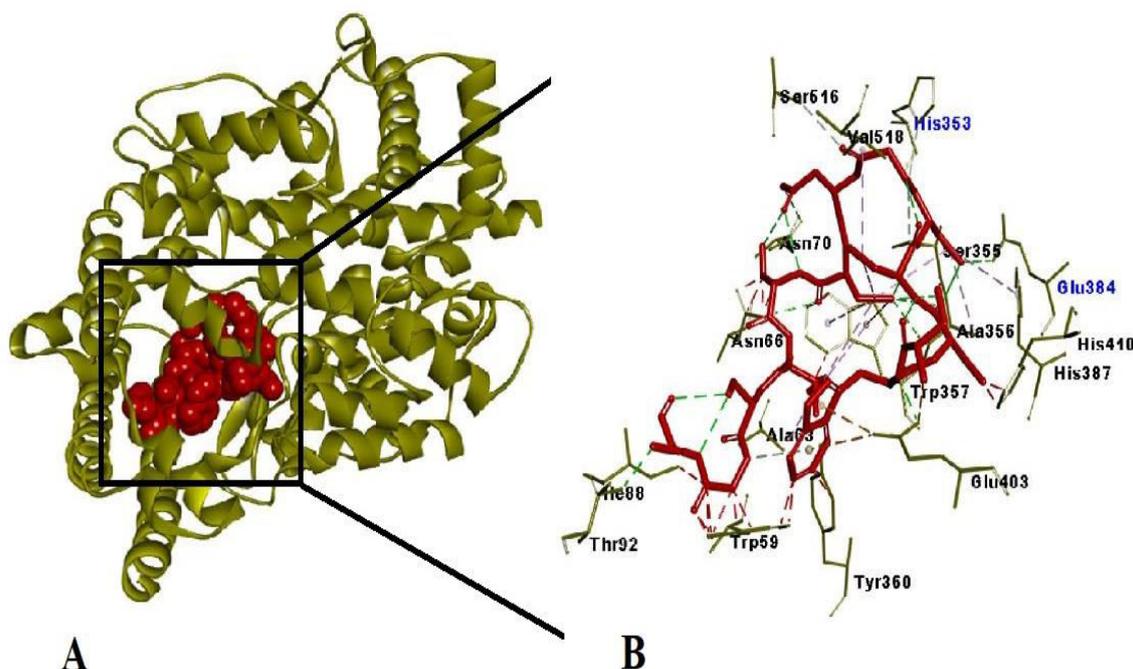


Fig. 3. Docked conformation of A) ACE-BL8 complex-I. The yellow ribbon structures indicate Angiotensin-converting enzyme (receptor) and the red space filling model indicates BL8 (Inhibitor) B) A close view of the different interactions involved in the complex. The red stick model indicates the inhibitor BL8 and the labelled amino acids are of the receptor protein

The amino acids involved in the binding pocket of 1O86 which binds to lisinopril, an ACE inhibitor, included Glu 384, His 353, Ala 354, Tyr 523, Tyr 520, Lys 511 [21]. Thus we can observe that both lisinopril and bacteriocin BL8 commonly bind to two amino acids in the binding site of ACE – His 353 and Glu 384, which once again adds to the substantiation that BL8 is an ACE inhibitor.

Protein–protein interactions are essential to cellular and immune function, and in many cases, in the absence of an experimentally determined structure of the complex, these interactions must necessarily be modelled for an understanding of their molecular basis [22]. The protein docking server ZDOCK, uses the rigid-body docking programs to predict the structures of protein–protein complexes [23]. This not only provides users with an accessible and intuitive interface, but also provides the options to guide the scoring and the selection of output models, in addition to dynamic visualization of input structures by BIOVIA Discovery Studio [24]. In this study, the C-value of the model protein BL8 was sufficient to provide the relevant information necessary for peptide design.

The amine group of the N-terminal Cysteine residue of BL8 established hydrogen bonds with the carboxylic group of Glu384 (Table 1, Appendix A). H-bond interactions established with Glu384 and Ser13 presented more potent interactions, which greatly contributed to stabilization [25]. This study thus provides inklings to the possible biological functions of BL8, which will be substantiated by other experiments. Thus application of bioinformatic tools enables the research community to easily and quickly produce structural models of protein–protein complexes and symmetric multimers for their own analysis leading to the Drug Discovery processes<sup>22</sup>.

#### 4. CONCLUSION

In this manuscript, we describe the extent to which Bioinformatic tools can be utilised in case of a protein whose atomic structure is still not solved and whose function is yet to be extensively investigated. We predicted distinguished features as well as the secondary structure of BL8, in addition to other structural features with neural networks-based methods. It was interesting to note that all the different prediction tools used here, with either the sequence or the structure as inputs, were in consensus in the results generated. This lends greater confidence in the approach used. An approach is taken towards the prediction of the most plausible protein–ligand complex from the complexes generated and in the event of little or no information available for the interface of the two binding partners. Structurally, BL8 protein domain seems to belong to alpha + beta protein fold family, whereas its interacting partner, ACE is an elongated all-beta structure. The question of the accuracy of complex prediction remains dependent on the experimental verification. On the whole our data and analysis supported the view that drug design can be aided by Bioinformatics tools and by protein modeling. In our study,

the E-value of the model was sufficient to provide the relevant information necessary for peptide design. This helps to save time and eliminate enormous costs due to random routine functional screens, making drug discovery and design easier.

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### SUPPLEMENTARY FILES

**Table 1:** Interaction table showing the interaction of the amino acids of ACE protein with the bacteriocin protein BL8. (Complex 1)

Interacting residues	Distance	Bond
A:SER1:N- A:GLU403:OE1	2.51279	Electrostatic
A:ASN66:ND2 - A:CYS8:O	3.19107	Hydrogen Bond
A:ASN70:ND2-A:SER9:OG	2.58521	Hydrogen Bond
A:THR92:OG1-A:SER13:OG	2.29898	Hydrogen Bond
A:ALA356:N - A:CYS5:SG	2.72172	Hydrogen Bond
A:ALA356:N - A:CYS8:SG	3.75053	Hydrogen Bond
A:TRP2:N - A:GLU403:OE1	2.18004	Hydrogen Bond
A:CYS4:N - A:SER1:O	3.0957	Hydrogen Bond
A:CYS5:N - A:CYS8:SG	3.18924	Hydrogen Bond
A:CYS5:SG- A:GLU384:OE1	2.57598	Hydrogen Bond
A:CYS5:SG - A:SER3:O	3.35831	Hydrogen Bond
A:GLY6:N - A:CYS4:O	2.82362	Hydrogen Bond
A:ASN7:ND2-A:ASN70:OD1	3.22574	Hydrogen Bond
A:CYS8:N - A:CYS4:SG	3.33851	Hydrogen Bond
A:CYS8:SG - A:ALA356:O	2.46252	Hydrogen Bond
A:CYS8:SG - A:SER3:O	3.45126	Hydrogen Bond
A:SER9:N - A:ASN7:OD1	2.97575	Hydrogen Bond
A:SER9:OG - A:ASN7:OD1	2.71684	Hydrogen Bond
A:SER11:OG - A:SER13:O	2.9139	Hydrogen Bond
A:SER13:N - A:SER11:OG	3.01142	Hydrogen Bond
A:HIS353:CD2 - A:CYS5:O	3.20681	Hydrogen Bond
A:SER355:CB - A:CYS5:O	3.53277	Hydrogen Bond
A:SER516:CB - A:GLY6:O	3.09963	Hydrogen Bond
A:GLU403:OE2 - A:TRP2	3.09448	Electrostatic
A:GLU403:OE2 - A:TRP2	4.01947	Electrostatic
A:GLY12:N - A:TRP2	3.76758	Hydrogen Bond
A:CYS8:SG - A:TRP357	4.27101	Other
A:ALA63 - A:ILE10	3.76191	Hydrophobic
A:ALA356 - A:CYS5	4.48997	Hydrophobic
A:CYS4 - A:VAL518	4.64264	Hydrophobic
A:CYS4 - A:CYS8	4.23183	Hydrophobic
A:CYS5 - A:CYS8	4.58299	Hydrophobic
A:CYS8 - A:ILE10	5.32705	Hydrophobic
A:TRP357 - A:ILE10	5.09342	Hydrophobic
A:TRP357 - A:CYS8	4.83437	Hydrophobic
A:HIS387 - A:CYS5	4.79971	Hydrophobic

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