Homology Modeling of Bacteriocins: From sequence alignments to structural models

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Abstract— Structural and functional characterizations of proteins have been one of the major problems in biological studies for a long time. The conventional methods of protein structure determination of NMR and X-Ray crystallography, though more accurate, are highly time consuming and tedious to carry out. Also, the instrumentation required is not that easily available. In the absence of proper structural information, like in the case of bacteriocins, which are anti-microbial proteins or protein complexes produced by lactic acid bacteria, comparative and homology modeling can be useful in predicting the structure based on the protein sequences and their alignment with one or more already known structures. The prediction process consists of fold assignment, target–template alignment, model building, and model evaluation. The present study focuses on the comparative protein-structure modelling of bacteriocins produced by five food starter cultures viz., *Pediococcus acidilactici*, *Leuconostoc mesenteroids*, *Enterococcus mundtii*, *Lactobacillus plantarum* and *Lactobacillus sakei.* The structures were modelled using MOE software and accuracy was evaluated based on the "errat" score. All the bacteriocins belonged to Class IIA subclass with same structural motif. The results of the present study will find its application in the develepoment of synthetic bacteriocins especially in the absence of structural information of such proteins.

Index Terms— Bacteriocins, Peptides, Homology Modeling, MOE, Errat

1 INTRODUCTION

Bacteriocins are proteins or protein complexes synthesised by various bacterial strains. They exhibit bacteriocidal and bacteriostatic activities usually against closely related species. Among these the most well studied one are the Class 2A which finds applications mainly in food preservation due to its efficient action against spoilage organism *Listeria monocytogenes*. It is also well known that, the lactic acid starter cultures of fermented foods display numerous antimicrobial activities which is mainly because of there ability to secrete a varity of bacteriocins. Another significant point of instrest are their antimicrobial activites against pathogenic microrganisms which have been reported by several workers[1],[2],[3]. Based on there structural and functional charactertsics they have been divided into three classes as tabulated in in table 1 below.

The mode of bacteriocidal action of these Bacteriocins is mainly by disruption of target membrane integrity leading to dissipation of proton motif force eventually leading to cell death [4]. Membrane located proteins of the manose-phosphotransferase system serve as target receptors for class IIA bacteriocin. Bacteriocins have specific cognate immunity proteins which binds to the receptor protects the host organism from the bacteriocin action [5].

Table 2 summarises the mode of action for the three classes of bacteriocins.

Table 1: Classification of Bacteriocins

CLASS I	CLASS II	CLASS III
Lantibiotics	Non modified heat stable peptides	Protein Bacteriocin
Type A: Elongated Shaped Molecule	2a: Pediocions like Bacteriocins	IIIA:Lysis causing
Type B:Globular Molecular	2b: Two peptide bacteriocins	IIIB

Table 2: Mode of action

CLASS I	CLASS II	CLAS S III	
Lantibiotics	Non modified heat stable peptides	Protein Bacteriocin	
Kill by distrupting the integrity of the membrane.	Kill by sensitizing the cell membranes	IIIA That kill bacterial cells by cell-wall degradation, thus causing cell lysis	IIIB Killing the target cells by disrupting the membrane potential, which causes ATP efflux .

2 METHODOLOGY

2.1 Selection of strains

Five strains and corresponding bacteriocins(*Pediococcus acidilactici*- Pediocin AcH[6], *Leuconostoc mesenteroides*-Mesenterocin Y105[7], *Enterococcus mundtii*- Munditicin[8], *Lactobacillus sakei*-Sakacin G[9], *Lactobacillus plantarum*-Plantaricin 423)[10]were screened out on the basis of the errat accuracy of the modeled structures of there bacteriocins. Errat is an online bioinformatics tool which can be used to verify the accuracy of protein structures. Structures with errat score of 80% above were selected.

2.2 Screening of Motif

A motif is a conserved region in a protein sequence which characterizes it as belonging to a particular class. The motif based search was also carried out for these bacteriocins using SCAN-PROSITE tool. The conserved class IIA motif "YGNGVXCXXXCXV" [11] was found out in all the sequenc-

es.

2.3 Screening for template structure

The template structures for these five bacteriocins were found out with the help of a sequence based BLAST search and the structure with the best combination of the query coverage and percentage similarity was chosen. The template structure for each of the bacteriocins is as represented below (Fig 1).

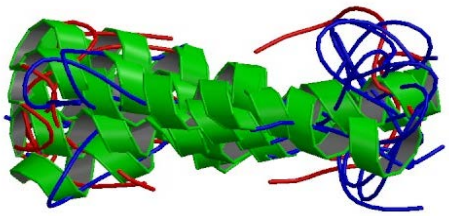

Fig 1(a)

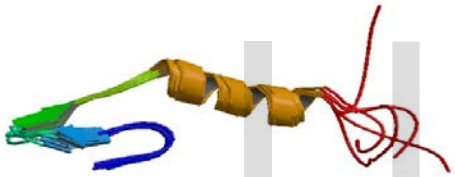

Fig 1(b)

Fig 1(a): SAKACIN P (PDB ID 1OHM): Template for Pediocin Ach and Munditicin. (b): (PDB ID 1CW6_A): Template for Mesenterocin Y105, Planatricin 423 and Sakacin G

2.4 Model Building

Based on the template structures and the sequence of the bacteriocins homology modeling was performed using MOE software.

3 Results And Discussions

Bacteriocins are well known anti-microbial agents which have been used as food preservatives [12]. However, one of the limting factors in the application of bacteriocin is their stability and efficacy in the food matrix. The protein stability studies are dependent on avalaibity of the three dimensional structures given by NMR and X-RAY crystallography techniques which may not always be avalaible. In silico based approaches provide a convienient method for the in depth studies and prediction of their functionality.

In this study we present the three dimensional homology models of five bacteriocins (Pediocin AcH, Mesenterocin Y105, Muditicin, Planatricin 423 and Sakacin G) (Fig 2). To the best of our knowledge these structures have not been reported earlier. The accuracy of the structures was evaluated using errat software (Verison 2.0) summarized in Table 3.

Table 3: Bacteriocins with the accuracy scores (percentage)

S.No	Bacteriocin	Errat accuracy score (%)
1	Pediocin AcH	80.00
2	Mesenterocin Y105	86.025
3	Munditicin	94.118
4	Planataricin 423	84.615
5	Sakacin G	100

The quality of the protein models is extremely important for the possible applications. As per the errat score, Sakacin G has the heighest accuracy of 100%. The model generated were further analyzed by bioinformatic tool, Conserved Domain Search, and were found to have the same structural domain (PFAM 01721) confirming they all belong to the class(IIA) bacterocin. The presence of same structural domain suggests that these bacteriocins may have a similar functional application. One of the best studied representatives of Class IIA is Pediocin AcH so much so that the class IIA is often also called as Pediocin-like bacteriocin [11]. Pediocin AcH is a very effective food preservative [12], [13], [14]. The various food matrices where Pediocin AcH has been applied are –Munster cheese (a variety of smear soft cheese) [15], chicken, smoked salmon [16]. Based on the above results of protein structure modeling, we further conclude that the other four bacteriocins might also have similar functional activity and stability in the above mentioned food matrices. Pediocin AcH has an anti microbial spectrum against *Lactobacillus, Leuconostoc, Pediococuss, Listeria monocytogenes, Listeria innocua, Listeria invanovi, and Clostridium botulinum, Carnobacterium, Enterococcus, Lactobacillus, Leuconostoc* and *Pediococcus*. It may be noted that the anti-microbial spectra of Plantaricin 423 and Sakacin G is not yet known. Considering the results of the modeling data presented in this study, it is indicated that these two may have the similar anti-microbial spectra. Further experimental studies would give an insight to the functional aspects of these bacteriocins.

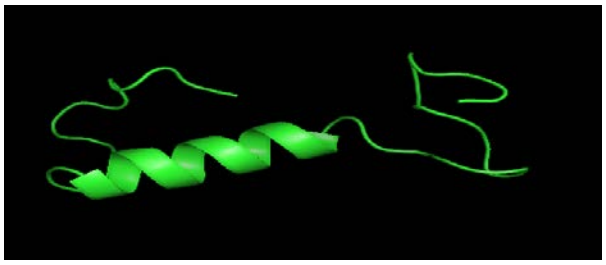

Fig 2(a) PEDIOCIN AcH

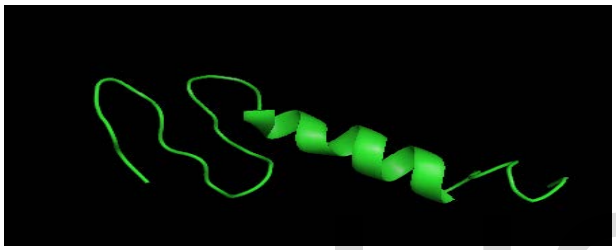

Fig 2(b) MESENTEROCIN Y105

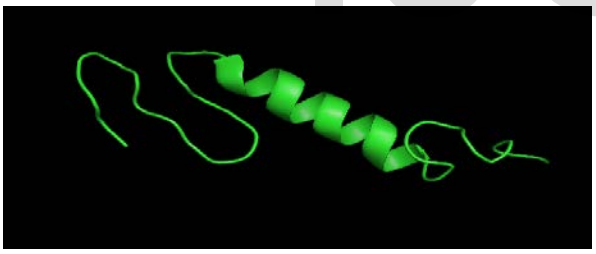

Fig 2(c) MUNDITICIN

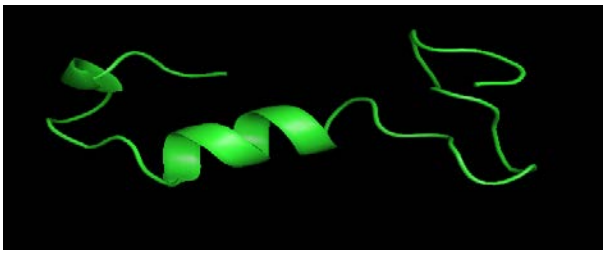

Fig 2(d) PLANATRICIN 423

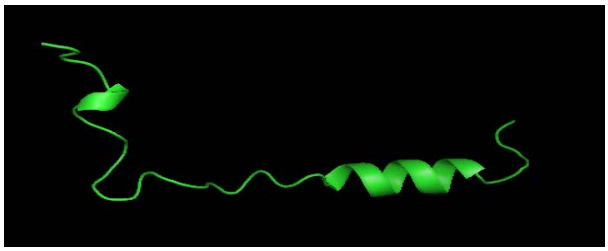

Fig 2(e) SAKACIN G

4 CONCLUSION

We present here the three dimensional homology of a very high accuracy based models of five bacteriocins which were not reported earlier. These structures can further be used to study the protein -protein interactions of these bacteriocins with various food components and study their stability in various food matrices. This kind of in silico approach helps in bringing down the cost and the time taken for such kind of efficacy studies. It is expected that within the next decade NMR spectrocopy will be able to provide us with a representive protein from each family hence increasing the applicability of these kinds of studies.

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