ORIGINAL ARTICLE

HOMOLOGY MODELLING OF PRION LIKE DOMAIN CONTAINING SINGLE STRANDED BINDING PROTEIN OF NOVEL BACTERIOPHAGE ATCEA85 INFECTING ENTEROBACTER AEROGENES: A STEP TOWARD UNDERSTANDING THE FUNCTION OF THE PRION LIKE PROTEINS

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ABSTRACT

Multidrug resistant bacterial strains have a great impact today causing serious diseases which are mostly difficult to treat. The use of illicit antibiotics led to the enhanced evolution of drug resistance. *Enterobacter aerogenes*, a bacterium is among these organisms. The usage of bacteriophages, viruses that infect bacteria has provided a relief in treatment strategies. Recently a novel bacteriophage infecting Enterobacter was identified to have a possibility of its use in therapy of Enterobacter infections. Prionome has been studied widely among the biosphere mechanisms. The current article aims at mining of the bacteriophage proteome for the presence of Prion like domain containing (PrD) proteins. A Single stranded binding protein was identified with PrD and the current study deals with homology modelling of the 3D structure of the protein which could provide a future platform to understand the mechanism of the protein at molecular level. PrD analysis of all Single stranded binding proteins from various bacteriophage species revealed the conservation of PrD association. These research findings will open a opportunity as of how these PrD proteins affect the physiology of bacteriophage infections

Keywords: Phage; Multidrug resistance; Pathogens; Antibiotics; Bacteria

INTRODUCTION

Enterobacter aerogenes is a bacterial species belonging to the ESKAPE group of pathogens and there is an exponential increase in the evolution of multidrug resistant strains of this pathogen species[1]. Hence the bacteriophages that could kill this pathogen are of utmost importance in the field of phage therapy. Many multidrug resistant strains of this pathogen are common, and it is responsible for the spread of carbapenem-resistance genes to other bacterial species. [2]. Due to the slower pace in development of new classes of antibiotics, the treatment has been a challenge with such evolved multi-drug resistant bacterial strains.

Bacteriophages are the viruses that evade bacteria. The use of phage to treat bacterial infections is one of the most evolving areas also commonly referred to as phage therapy. Even some phage derived products, mostly endolysins along with phages are used in place of antibiotics. Recently, a novel phage that infects the *Enterobacter aerogenes* is reported[3]. Bacteriophage host range is quite narrow and once a bacterium is prone to bacteriophage infection, a bacterial mutant could arise. And appending this, the phages also mutate quickly to adapt to the mutant bacterial strains[4]. Enterobacter phage ATCEA85 belongs to the order *Caudovirales* and Family *Siphoviridae*. Prion like proteins were proved to have key roles in eukaryotic and prokaryotic organisms[5]. Prion proteins (PrPs) are self-propagative and can switch between confirmational changes as required in the environment and new prions evolve in this way[6]. The well-known form of PrPs are the amyloid proteins which are infectious and besides involved in various physiological processes in eukaryotes and prokaryotes[7]. Even though the sequence and functions of these evolutionary different proteins are variant, all these amyloid fibrils are misfolded into distinct conformers which form highly ordered cross-β structures[8].

Recently published studies established that this ordered parallel or antiparallel amyloid β sheets were found in bacteria and yeasts indicating key physiological roles[9]. These amyloid sheets have variant functions in bacterial species and the secretion of this amyloid proteins is a key step in biofilm formations[10]. Hence the expression of prions was related to enhanced environmental adaptation, stress response activation, and in long term memory in yeast[11]. Since these PrPs are found to have abundance of glutamine (N) and asparagine (Q) domains, the same is used to screen proteins for potential prionogenic domains (PrDs)[12]. The currently used PrD prediction algorithms are based on hidden Markov model (HMM), which is based on the probabilistic sequence that uses maximum likelihood estimation[13]. HMM is key among many of the approaches in bioinformatics for the statistical representation of prion domains and their scoring to prove that they are prions[14]. Using these online algorithms, the prion-like domains from mammals, bacterial and fungal proteomes were studied which were important global regulators.

Prion like domains in bacteriophages, have not been fully characterized. In the current study we screened the proteome of a novel bacteriophage ATCEA85 for the proteins with prion like domains. We found that one among these proteins which is a Single stranded binding (SSB) protein has a prion like-domain potentially and is having key role in the multiplication of bacteriophage ATCEA85 in infecting the *Enterobacter aerogenes*. Modelling of the structure of such proteins would pave a way to understand better the functions of proteins.

MATERIALS AND METHODS

Protein Sequences

To analyze the PrDs present in the proteome of novel bacteriophage ATCEA85, protein sequences were retrieved from Uniprot Knowledge Base (Swiss-Prot and TrEMBL), (UniProt release 2021_03). The functions of the protein sequences were obtained manually from the Uniprot KB. NCBI Nr database and literature databases.

PrD analysis in proteome of bacteriophage ATCEA85

All the proteins from the proteome of novel bacteriophage ATCEA85 were analyzed using PLAAC Server (Prion-like Amino Acid Composition) which is a tool that does prediction using HMM. The tool was designed with trained sets with known PrDs which harbour N and Q residues. To cross verify the PrDs in the same family of proteins, various Single stranded binding (SSB) protein sequences from bacteriophages.

Homology modelling of the structure of SSB from bacteriophage ATCEA85

The 3D structure of SSB protein from bacteriophage ATCEA85 was predicted using iTASSER (Iterative Threading ASSEmbly Refinement) server with default parameters. Besides prediction of the 3D structure of the iTASSER also predicts the possible ligand binding sites on the protein which could be used for future docking studies.

Evaluation and Refinement of the model

The predicted model was evaluated for the structural integrity using Ramachandran plot derived for the structure. This was done using Saves tool at UCLA server. The misconfigured residues were then refined through loop modelling in SPDBV v 4.1.0 tool. The structure energy was minimized using 100 iterations of Steepest Descent gradient and 10000 cycles of Conjugate gradient in SPDBV tool. The final model was once again evaluated on SAVES tools.

RESULTS

PrD analysis in proteome of bacteriophage ATCEA85

For identification of PrDs in bacteriophages using PLAAC server, a total of 46 protein sequences from proteome of Enterobacter phage ATCEA85 with proteome ID UP000427602 were retrieved from UniProt KB database. One protein with Uniprot ID A0A649V373 has a Prion like Domain of 41 Aminoacids (Figure 1). This protein is a single stranded DNA binding protein. To identify if PrDs are present only in this single protein or in the whole family of Single stranded DNA binding proteins, all the Single stranded DNA binding proteins from various bacteriophages were retrieved from the UniprotKB database. A total of 179 single stranded DNA binding proteins were used for this analysis. Most of these proteins have found to have PrDs. Besides, a Phylogenetic tree was constructed from these sequences to investigate the evolution of this protein reveals the conservation of the Single stranded DNA binding proteins among bacteriophages.

Homology modelling of the structure of SSB from bacteriophage ATCEA85

iTasser prediction resulted in various models, among which model 1 was chosen which was predicted with higher score. This model was evaluated using SAVES tool, Ramachandran plot revealed that the Amino acid residues Lys 63, Lys 74, Arg 97, Tyr 98, Glu 101, Val 102, Val 110, Asn 124, Asn 144, Gln 154, Asp 165 were having wrong dihedral angles (Figure 2) and the dihedral angles were corrected through loop modelling and energy minimization using SPDBV tool. The Ramachandran plot of the final refined model showed all the residues rectified or remodelled (Figure 2). The initial model and the final refined structure of the final model is represented in (Figure 2).

DISCUSSION

This study reports the PrD like Single stranded DNA binding proteins in the novel bacteriophage ATCEA85. The results of the current study state the importance of analysing the bacteriophage proteomes for PrDs and modelling of the 3D structures of such PrDs which form most important components of Phagobiome as reported earlier[15]. PrDs are frequently observed in Archaeal phages and the previous studies represent the presence of PrDs in families of *Caudovirales* and it is hypothesised that 50% of sequence bacteriophages atleast have one PrD[15]. Since the proteins in *Siphoviridae* are lesser in number compared to that of *Myoviridae*, there are lesser number of PrDs in *Siphoviridae*. Majority of the bacteriophage PrDs are involved in functions such as bacterial cell wall attachment, penetration and release of progeny. This study states the possible role of the Single-stranded- binding protein in the regulation of replication of phage. The phylogenetic analysis of the various Single-stranded- binding protein reveals that PrDs are conserved across various species of bacteriophages and are known to have various regulatory roles. Previous studies reported the role of PrDs could alter their conformational states and may enhance the specificity of phage-bacterial interactions[15]. It is also hypothesised that the type of interaction the PrDs have with host cell is one of the additional mechanisms by which the phages control the bacterial intracellular functions. Considering all these aspects, it is very essential to understand the function of the bacteriophage proteins with PrDs. In order to that, a 3D structure is very essential which we attempted in this research paper to generate a modelling approach which could be used in future to study the phage proteins harbouring PrDs.

CONCLUSIONS:

The Single-stranded- binding protein of Enterobacter phage ATCEA85 is found to harbour a prion like domain (PrD) which makes it a protein involved in regulatory role. The modelled 3D structure of this protein would pave a way to better understand the molecular interaction of the PrD in the bacteriophage cycle. To our knowledge this is the first study of PrD in the proteome of Enterobacter phage ATCEA85 which has a key role in combating multidrug resistant strains of *Enterobacter aerogenes*.

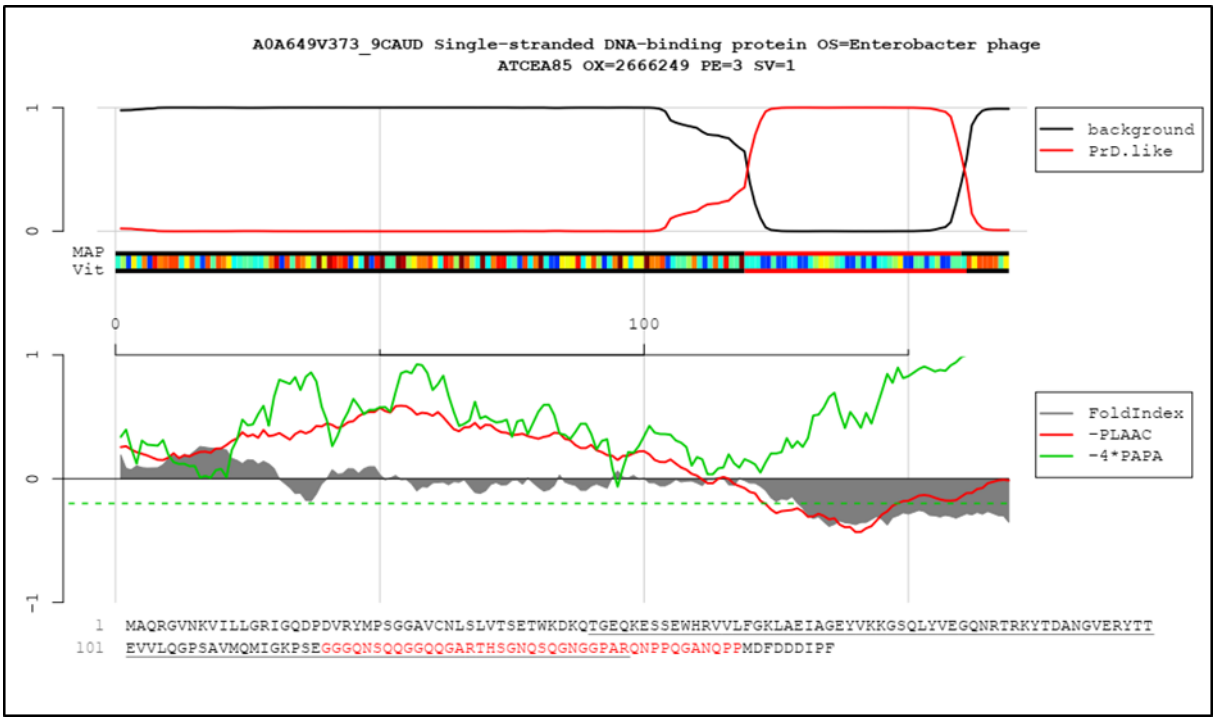

Figure 1: Prion like Domains predicted from PLACC analysis of Single-stranded binding protein of Enterobacter phage ATCEA85

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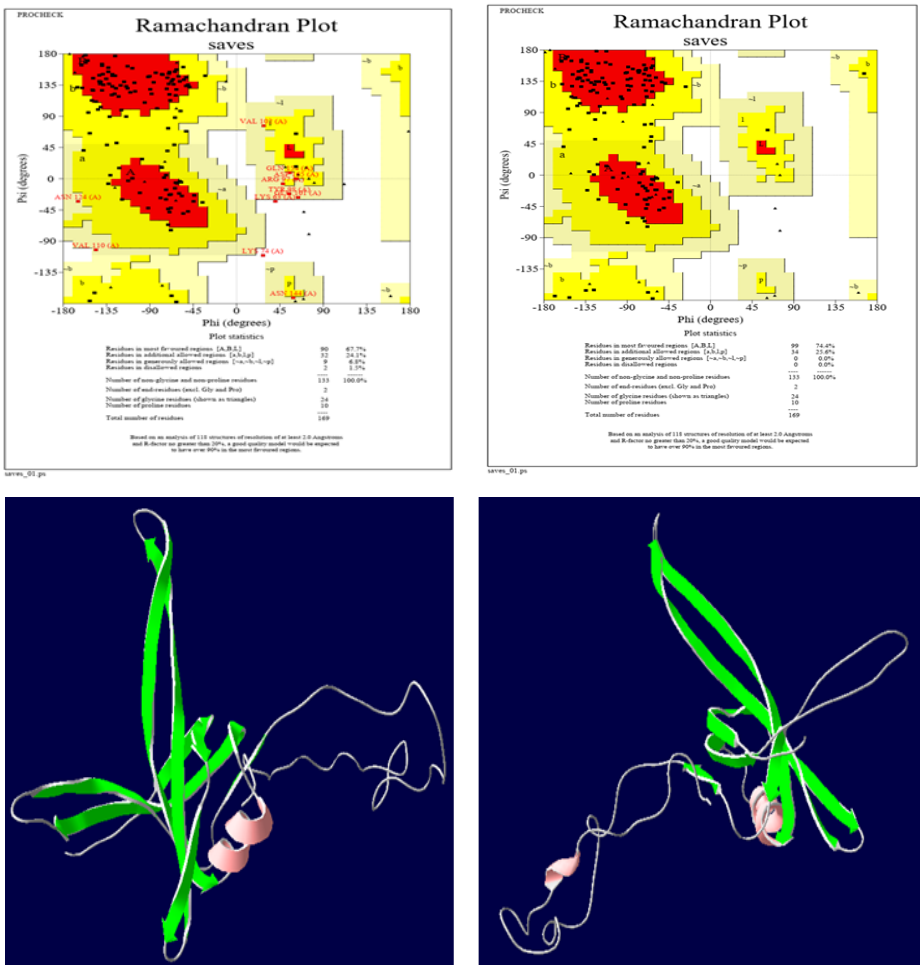

Figure 2: A) Ramachandran plot of initial model obtained from iTASSER server (Unrefined) B) Ramachandran plot of refined model of the study C) Initial model structure D) Final refined model structure showing cross β-structures

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