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RESEARCH ARTICLE

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Mycosubtilin: 3D protein structure prediction and its extensive study as a biosurfactant

Sabyasachi Mohanty * and Sarbani Mishra

Department of IMSc Bioinformatics, BJB (A) College, Bhubaneswar, India

For correspondence: sabyasachi.mohanty22@gmail.com

ABSTRACT

The surfactants from the microbial origin that can be synthesized by several identified microorganisms including bacteria, yeast, and fungi are known as biosurfactants. Excellent surface activity and emulsification properties with very low toxicity and higher biodegradability features as compared to chemical counterparts have been observed in them. This paper deals with the structure prediction of a biosurfactant protein mycosubtilin whose structure was unknown, using the geno3D server which was validated using the SAVeS server. This protein has some antibiotic properties along with antibacterial and antifungal properties. Afterwards, the extensive study of the mycosubtilin protein as a biosurfactant was studied, including its reactivity with a metalloprotein metallotheonein.

Keywords: Biosurfactant, mycosubtilin, metallotheonein, homology modelling.

1. INTRODUCTION

Biosurfactants are a group of surface-active molecules with hydrophilic and hydrophobic moieties conferring the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively [1-4]. Biosurfactants are usually produced by a diverse population of microbes such as bacteria, fungi, and yeast [5-7]. They are mainly classified according to their chemical structure and their microbial origin. Chemical surfactants could be replaced with biosurfactants and this change would diminish the environmental impact of traditional dispersants [8-10].

Biosurfactants are found to have not only antimicrobial properties towards various microorganisms but are also involved in cell adherence which imparts great stability under hostile environmental conditions and virulence and in cell desorption when organisms need to find habitats for survival [11-13]. Here we are dealing with a protein that had no structure, but acts as a biosurfactant i.e; mycosubtilin. Its

structure was designed using the homology modelling method. The protein mycosubtilin belongs to the iturin family of lipopeptide antibiotics [14]. The lipopeptides belonging to iturin family are macrocyclic and possess antimicrobial, antifungal, antibiotic, and surfactant properties [15-17]. But the problem lies in the fact that the enzymes responsible for the biosynthesis of the iturin lipopeptides are still unknown.

2. METHOD AND MATERIAL

2.1. Identification of proteins responsible for biosurfactant production

The determination of the 3D structure of the protein mycosubtilin using the homology modelling method was used. The protein sequence was extracted from the strain *Bacillus amylolequifaciens* from the UniprotKB database.

2.2. Searching for the template search and model designing

The Geno3D server is used for homology modelling. The target sequence from Uniprot was submitted in FASTA format without the description line to search for templates. For each template, the Geno3D server computes secondary structure prediction, displays percent of agreement in secondary structure, repartition of information from the template on query sequence. The working mail address should be entered as the resulting models will be sent via mail. Distances and dihedral angles restraints calculated from the alignme--nt with the templates 3D structure are applied on query sequence. For gaps, statistical restraints are used.

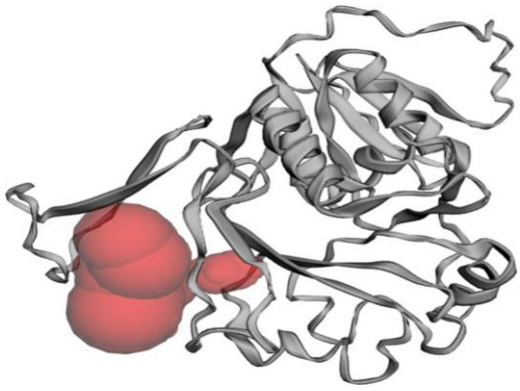

Figure 1. Visualisation of the pocket in mycosubtilin protein

The output is 3D models that satisfy these restraints as well as possible. At the end of the molecular modelling process, a mail was received which provided molecular modelling results. A total of 10 models were generated for mycosubtilin.

The model with the minimum potential energy was chosen for further studies. To view the model generated by homology modeling the visualization tool Rasmol (a molecular graphics program) was used.

2.3. Protein structure analysis and validation

The protein is validated and evaluated using the SAVeS (Structure Analysis and Verification) SAVeS possesses tools such as PROCHECK, WHAT CHECK, ERRAT, VERIFY 3D and PROVE. Ramachandran plot was also generated by the server. The PDB file was uploaded, and all the programs mentioned above were selected to run the programs.

2.4. Study of the reactivity of mycosubtilin protein with metalloprotein

The binding mode and binding affinity of a complex formed by two or more constituent molecules with known structures were studied through molecular docking which is used widely to predict the conformation of ligand-receptor complex. Hence protein-protein docking has been done to study the interaction between the receptor mycosubtilin and metallotheonein.

2.5. Steps in docking

To increase the docking efficiency the active site of the receptor was recognized using the CASTp (Computed Atlas of Surface Topography of proteins) server to detect the active site of the receptor protein mycosubtilin. The coordinate

file of a structure in PDB format is to be submitted to the CASTp server. When the calculation is finished, a visualization window will be opened.

2.6. Docking methodology

To study the protein-protein interaction between mycosubtilin protein and metallotheonein the docking software HEX was used, which is an interactive protein docking and molecular superposition program. The mycosubtilin model generated from the geno3D server was loaded along with the metallotheonein model from PDB. Then all the parameters were set from the control menu and proceed the docking procedure. The result of docking was analyzed afterward which was visualized under the pymol.

3. RESULTS AND DISCUSSION

Bacteria of genera *Pseudomonas* and *Bacillus* are found to produce biosurfactants but due to their pathogenic nature they could not be used in food industries. The most commonly studied yeasts for biosurfactants will be sent via mail. Distances and production are *Candida bombicola* and *Candida lipolytica* [16-17]. There are also many biosurfactant proteins whose structures are undetermined eg; lichenysin, serrawettin, aneurinifectin, mycosubtilin, etc. The protein sequence was extracted from the strain *Bacillus amylolequifaciens* from the UniprotKB database. The sequence length was found to be 298.

3.1. Result of Homology Modelling

Homology modelling refers to the modelling of a 3D protein structure based on the known experimental structure of its sequence homologous (figure 2). It is a multi-step process that includes sequence alignment, structural modification, database searches, energy minimization, and structure evaluation to generate a structure. Homology modelling was retrieved Uniprot sequence of mycosubtilin (Uniprot Accession ID)[19] is then put in a homology modelling server to start modelling. A total of 10 models were generated from the geno3D server of which the model with the

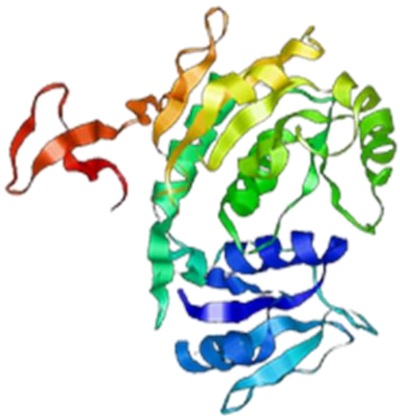

Figure 2. The 3D model of mycosubtilin protein visualised under Pymol

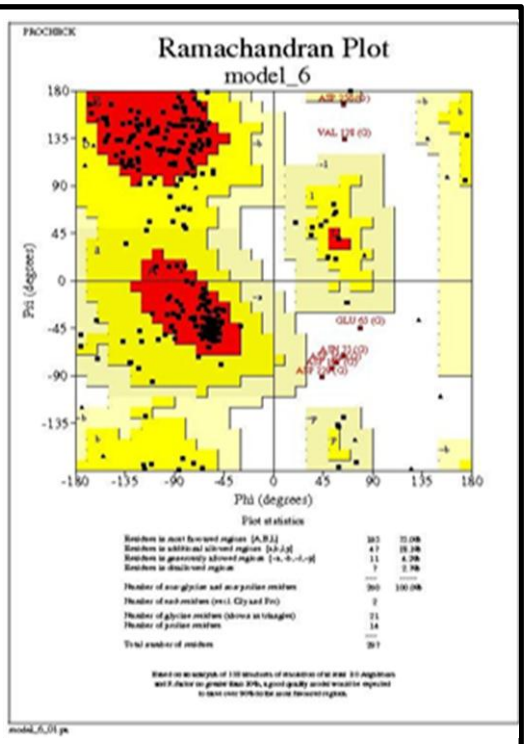

Figure 3. Ramachandran plot for the mycosubtilin protein

minimum energy i.e., -12445.30 kcal/mol was chosen. According to molecular dynamics studies, it is known that a protein molecule in its native state having the least potential energy is the most stable structure [20-21].

3.2. Ramachandran plot generated from SAVES server

The Ramachandran plot visualizes the dihedral angle φ (phi) against Ψ (psi) for amino acids in the protein structure (figure 3). The red regions depict the conformations where there are no steric clashes, i.e., these are allowed regions namely the α-helical and β-sheet conformation [22]. The yellow areas show the allowed regions if slightly shorter VanderWaals radii are used in the calculation i.e., the atoms are allowed to come a little closer together [23-24]. This brings out an additional region that corresponds to the left-handed alpha-helix. The white areas are conformation where atoms in the polypeptide come closer than the sum of their Van der Waals radii. These regions are sterically disallowed for all amino acids except glycine which is unique in that it lacks a side chain. Hence from the above Ramachandran plot, it is clear that the resulting model of the protein mycosubtilin is structurally correct [25].

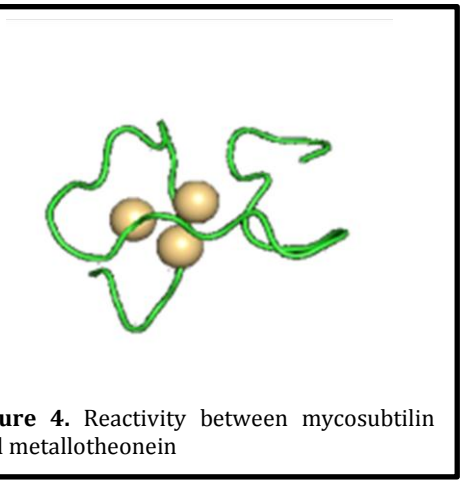

Figure 4. Reactivity between mycosubtilin and metallotheonein

3.3. Docking result

The binding energy for these molecules was calculated. The binding energy profile of mycosubtilin protein and metallotheonein indicates that they bind to each other with a binding energy of -5.68 kcal/mol (figure 4). An added feature for the calculation of uploaded structures is that the user can adjust the probe radius to any values between 0.0 and 10.0 Å [26].

4. CONCLUSION

Here we did the prediction of an unknown protein model of mycosubtilin, by the geno3D server which is a homology modelling tool. The model with the minimum potential energy of -12445.30 kcal/mol was chosen to be the best model with maximum stability. Further studies

on this protein reveal that it can be used as an antifungal, antibacterial agent and it also possess some antimicrobial properties. The reactivity of the biosurfactant protein mycosubtilin with metalloprotein metallothionein was observed by performing docking in HEX, which gave us binding energy of -5.68 kcal/mol.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

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