Journal of Advanced Bioinformatics Applications and Research
ISSN 0976-2604.Online ISSN 2278–6007
Vol 5, Issue1, 2014, pp28-32
http://www.bipublication.com

HOMOLOGY MODELLING OF PBP2a PROTEIN FORM METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS*

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[Received-13/03/2014, Accepted-20/03/2014]

ABSTRACT

Homology modeling aims to build three-dimensional protein structure models using experimentally determined structures of related family members as templates. SWISS model workspace is an integrated Web-based modeling expert system. In the current research the 1279 base pair nucleotide of *mecA* gene which was sequenced and search against open reading frame tool and the tool was searched six open reading frames, the maximum length of 366 amino acid residue sequence was chosen for homology modeling of PBP2a protein. The 366 amino acid PBP2a was aligned with 1VQQ, 1MWS and 1MWR PDB sequences with pair wise sequence alignment the maximum alignment was shown with 1VQQ PDB sequence and 1VQQ sequence was considered as a template structure for homology modeling of PBP2a protein. Using SWISS model the three dimensional structure was generated and verified with verified tool. The verified tool was used to assess and estimate the reliability of the resulting models.

Key words: *PBP2a protein, Swiss model, PDB database, ORF finder. PDB, S. aureus*

[1] INTRODUCTION:

Homology modeling of protein refers to constructing an atomic three-dimensional model of the target protein from its amino acid sequence to generate three-dimensional structure of a related homologous protein. Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence [1]. To select templates for a given protein, the sequences of the template structure library are searched [2]. The PDB database was searched for suitable templates and 1VQQ, 1MWS and 1MWR are found to be suitable PDB structure for homology modeling of PBP2a protein. Among three structures 1VQQ was most suitable structure because which was give maximum alignment score with our template structure.

[II] MATERIALS AND METHODS:

2.1 Pair wise analysis of sequence:

Pair wise Sequence Alignment was used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences. The 366 amino acid PBP2a protein sequence was aligned with 1MWS, 1VQQ and 1MWR PDB sequences, these are the class of PBP2a (Penicillin Binding Protein 2a) proteins. The EBI EMBOSS- Needle sequence alignment program was used for pair wise alignment

2.2 Template selection for PBP2a:

The PDB (protein data bank) is the large collection of 3D structures for macromolecules which include protein, peptides, nucleic acids, carbohydrates and other bio-molecules. After analysis of pair wise sequence alignment with 1VQQ, 1MWS and 1MWR PDB sequences, the 1VQQ showed maximum homology towards our protein sequence (PBP2a). Hence, PDB structure with PDB ID (ID- Identification Number) 1VQQ selected as a template structure for homology modeling.

2.3 Homology modeling for PBP2a protein:

To generate 3D structure of PBP2a protein (homology modeling) which was responsible for antibiotic resistance in *S. aureus* PBP2a (Penicillin Binding Protein 2a). The SWISS-PDB software was used; it is fully automated protein structure homology-modeling server. SWISS PDB software was downloaded for modeling of PBP2a protein structure. The 1VQQ structure was downloaded from protein data bank (PDB) as a PDB file format. Using file option open the PDB file (1VQQ structure) loaded on to the software, after displaying the 1VQQ structure on the software, using the option open Swiss model, load the raw sequence on to the software (366 amino acid residue protein sequence). Open the option magic fit which is at option fit, the magic fit was used for the super imposition of raw amino acid sequence with 1VQQ structure and lastly the option update threading display was used and this option were present at SWISS model option for the generation of 3D model. The generated 3D structure was further analyzed by Rasmol visualization software.

2.4 Verification of 3D model:

The generated 3D structure need to be verified for its quality. To verify the structure generated from homology modeling based on analyzing the compatibility of an atomic model (3D) with its own amino acid sequence (1D). Each residue is assigned a structural class based on its location and environment (alpha, beta, loop, polar, non-polar, etc). Geometrical parameters of a given structure are obtained including: local backbone geometry class, burial status of all residues (based on the number of inter atomic contacts with other residues), and contact distances between different residues calculation of local, burial and contact energy is accomplished by application of statistical potentials of mean force to these parameters, in many cases PSQS (protein structure quality score) makes it possible to differentiate between correct and incorrect protein structures. It should be noted, however, that essentially PSQS reflects the similarity between a given protein structure and a typical protein structure. PSQS may be used in homology modeling to evaluate alternative protein models based on different alignments. It may also be useful during structure refinement to locate the places that are significantly different from typical geometries. For the verification of PBP2a structure, VERIFY 3D structure evaluation Server was used and also model was sent for JCSG (Joint Center for Structural Genomics) for further verification.

[III] RESULTS:

3.1Pair wise sequence analysis:

The pair wise sequence analysis was used to identify the similarity of PBP2a (366 amino acid

residue sequence) towards the existing sequences in the various databases. The 1VQQ PDB sequence showed maximum similarity of 55.9% with ORF sequence comparatively other two sequences 1MWS (55.7%) and 1MWR (54.5%) respectively. Hence, the 1VQQ PDB sequence/structure was used as a template for construction of the 3D model.

3.2Homology modeling for PBP2a protein:

Homology modeling of the structure was based on the template structure. The template structure must have some percentage of similarity and this was searched using protein BLAST choosing option as a protein data bank (PDB) by taking ORF as query sequence. The 1VQQ, 1MWS and 1MWR are homologous structures available in the PDB database. Based on the sequence and structural similarity towards ORF sequence, the PDB ID 1VQQ structure (Fig: 1) was selected as a template for homology modeling. The homology modeling using SPDB-Viewer software [3], the template structure of 1VQQ and PBP2a raw sequence was loaded on to the software using align option both structure and sequence was super imposed and finally using the option magic fit structure was super imposed and 3D structure was predicted for PBP2a protein (Figs: 2 and 3).

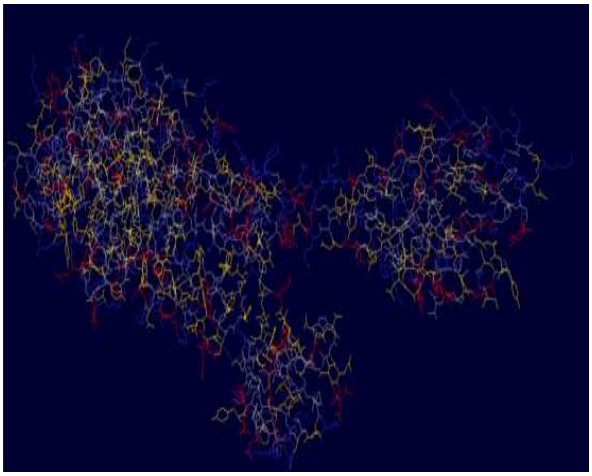

Fig-1: 1VQQ Chain-A- 3 Dimensional Structure of penicillin binding protein (PBP2a)

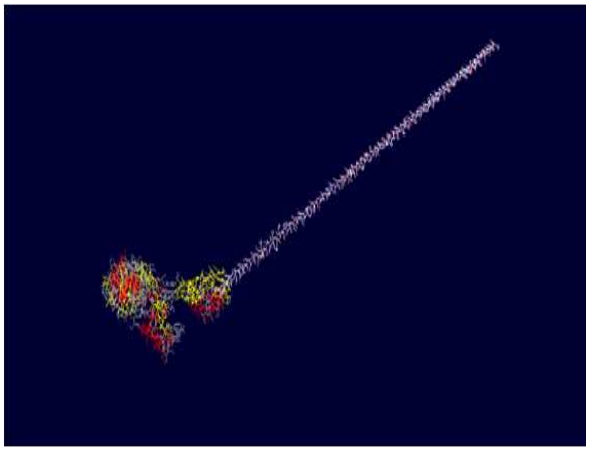

Fig-2: Penicillin binding protein (PBP2a) structure from PDB database with PDB ID 1VQQ and raw sequence of modeling protein (Template structure and modeling sequence on Spdb-Viewer software)

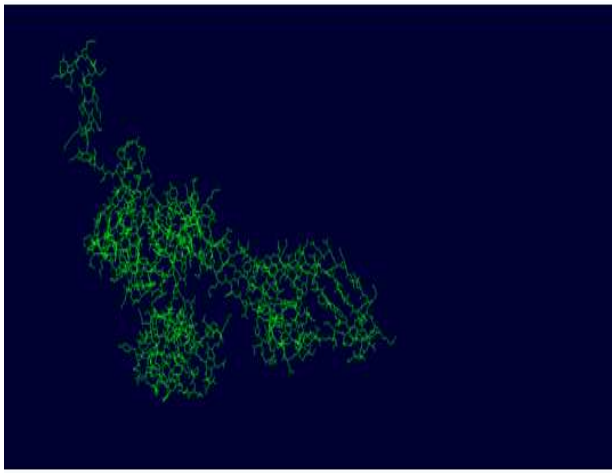

Fig-3: 3D (Dimensional) Structure of model penicillin binding protein (PBP2a)

3.3Verification of 3D Model:

The generated PBP2a structure verification by verify 3D structure evaluation server showed above Zero value and it confirms as a good structure [4]. (Fig: 4). The same structure was further evaluated with Joint Center for Structural Genomics (JCSG) for Protein Structure Quality Score (PSQS). The graph shows the distribution of PSQS (Protein Structure Quality Score) potential along amino acid chain. Crosses correspond to PSQS values obtained from

calculations. The curve shows running average of values calculated for a given amino acid and its two neighbors to the left and two neighbors to the right. Average PSQS for PDB structures falls between -0.1 to -0.3 [5]. The generated model of PBP2a structure total score were showing -0.1551(Fig: 5) and the model PBP2a structure was within the range of average PDB PSQS structure value (-0.1 to -0.3).

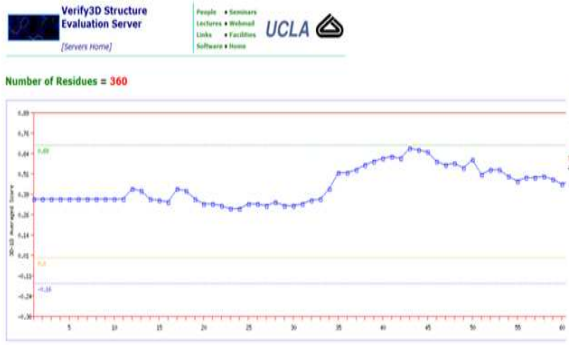

Fig- 4: Verified 3-D graph of PBP2a protein structure shows above zero value confirming prediction

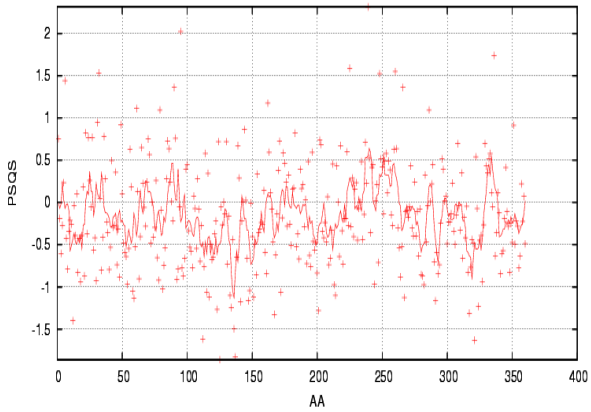

Fig-5: PSQS (Protein Structure Quality Score) value of model PBP2a structure

No.	Local	Burial	Contact	Total
1	-0.0213	-0.0755	-0.0584	-0.1551

[IV] DISCUSSION:

Homology modeling is the determination/prediction of 3D structure using sequence information [6]. In our investigation, after identifying the PDB ID1VQQ structure as a homologous structure for PBP2a protein and same was selected as a template for the prediction of 3D structure [7].

The SPDB-viewer is the choice of software for the prediction of 3D structure because; software is user friendly for the prediction of complex structures. Prediction of tertiary structure of a protein molecule signifies an important step towards understanding the structure–function relationships in the concerned protein family [8]. In our study the PDB ID 1VQQ PBP2a protein template structure was loaded on to the software and the raw sequence of 366 amino acid sequence was loaded using the option load raw sequence, using the option super impose the sequence and structure was super imposed and using the option magic fit the 3D structure was predicted and predicted structure was further evaluated. Recently, the first solution structure of a LEA protein, LEA14 from *Arabidopsis thaliana* has been reported by [9]. Another report says that [10], the three–dimensional model of the *rpoB* protein (NP_215181) is generated based on the crystal structure of the *Thermus thermophilus* (PDB: 2CW0). The other report evident for the prediction 3D structure using BLASTP for identification of the most suitable template for homology modeling of NS3 protein. The available structure of NS3 protein from MVEV in the protein database (PDB entry 2WV9) was used as a template for DENV, JEV, USUV and WNV. The homology modeling was carried out using the Modeler 9v7 program [11].

The prediction of 3D structure from raw sequence using homology modeling approaches further need to be verified for the generated 3D structure [12 and 13]. Because all the 3D structure available in the PDB structure database, the average PSQS (Protein structure quality score) will be between -0.1 to -0.3 [5]. The predicted structure score between these ranges were

considered as better structures. In our current study for verification of model has done with tools VERIFY 3D Structure evaluation server and Joint Center for Structural Genomics (JCSG). The score for model at VERIFY 3D was above zero value (-0.39) (Fig: 40a) and for tool Joint Center for Structural Genomics (JCSG) was -0.1551 these scores are indicating the generated structure was better for further studies. The previous study reports on the structure modeled and verified using VERIFY 3D tool for HIV-1 PR and the result value was above zero interpreted by [4].

[V] CONCLUSION:

In our current study by using Bioinformatics tools we have predicted 3D structure for PBP2a protein, using 1VQQ as a template structure. Based on the template structural information the 3D structure was predicted for PBP2a protein. The process of prediction of 3D structure using available template structure is known as homology modeling. The predicted structure was verified with VERIFYD 3D and Joint Center for Structural Genomics (JCSG) tools, the results obtained was -0.39 and -0.1551 respectively. The score was above the zero value (for standard structure validation) and structure was used for futher *In silico* docking analysis.

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