INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-8, Issue-3, July-Sept-2017 Coden IJABFP-CAS-USA Copyrights@2017

Received: 12th May 2017 Revised: 8th June 2017 Accepted: 9th June 2017

DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt Research article

MODELING AND DOCKING STUDIES OF ANTIBACTERIAL PEPTIDE 50S RIBOSOMAL PROTEIN L31 EXPRESSED IN FRESH WATER CRAB BARYTELPHUSA GUERINI

Mehad Mustafa[1], Nadeem Fatima Ansari[2],Jayasimha Rayalu Daddam[3]., and N.V.Naidu[4]

[1]Department of Chemistry, Faculty of Science and Technology, Mewar University, Chittorgarh (Rajasthan) India.

[2]Anwar Ul Uloom College of Arts, Science and Commerce, Mallepally, Hyderabad, Telangana State, India

[3]Department of Biotechnology, Dr.Rayalu's Biotech PVT.LTD., Himayath nagar, Hyderabad, Telangana, India

[4]Global Institute of Biotechnology, 3-6-276/2, Himayat Nagar, Hyderabad-500029.T.S. India.

ABSTRACT: Homology modeling has significant potential as a tool in Bioinformatics, in particular in high throughput in silico screening. In this work, Proteomic approaches were applied to separate proteins by using 2D-PAGE, (Two-Dimensional Gel Electrophoresis) and proteins were identified using MALDI-TOF-MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry). From the MS data and peptide matching, it was identified that the protein expressed was 50S ribosomal protein L31 from Barytelphusa Guerini. We have studied the expression, regulation, and protein analysis of 50S ribosomal protein L31. After the peptide sequencing, domain and function of the protein was identified. 50S ribosomal protein L31 structure was generated using MODELLER9V7 software using 2AW4 as a template. With the aid of the molecular mechanics and molecular dynamics methods, the final model was obtained and further assessed by PROCHECK and Verify 3D graph programs, which showed that the final refined model is reliable. With this model, a flexible docking study was performed with Sortase A of staphylococcus aureus using GOLD3.0.1 software. The results indicated that ARG 157, THR115, LEU242 in Sortase A are amino acids involved in docking studies as these were having bonding with the Sortase A.

Key words: Barytelphusa Guerini, 2D-PAGE, MALDI-TOF-MS, Homology Modelling, Ribosomal protein L31.

*Corresponding Author: Mehad Mustafa' Department of Chemistry, Faculty of Science and Technology, Mewar University, Chittorgarh (Rajasthan) India.

INTRODUCTION

Ribosome is a molecular machine that synthesis protein according to the genetic information presents in nucleic acid (Wimberly et.al 2000, Schluenzen et.al 2000). Ribosome is responsible for catalyzing and synthesis of the different ribosomal components is likely to occur, in response to unfavourable environmental conditions for the transcriptional activity of the cell. rRNA synthesis has been found to be rapidly down regulated in different stressful situations, and is more efficiently down regulated than overall mRNA synthesis (Mayer et al., 2005). The overall the processes of transcription, translation and assembling with ribosomal proteins, are very sensitive to cellular stress (Olson, 2004). Ribosomal proteins are present in stoichiometric amounts in ribosome (Wittmann1986). Some ribosomal proteins have special ribosomal functions, besides ribosome structure and protein biosynthesis (Chen and Ioannou, 1999), As ribosomes are protein-rRNA complexes, this also implies a high degree of amino acid sequence conservation between equivalent ribosomal proteins in different species (Draper and Reynaldo, 1999). The ribosomal proteins of *Escherichia coli* were systematically characterized as S1 to S21 for the 30S subunit and L1 to L34 for the 50S subunit, on the basis of their behaviour in two-dimensional gel electrophoresis (Kaltschmidt *et.al* 1970, Wittmann, *et.al* 1974, Wittmann *et.al* 1971, Wittmann *et.al* 1980).

The structural gene identified for ribosomal protein L31 is rpmE (Eric 1981). Generally protein structures are typically determined by experimental approaches such as X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. The high resolution structures of both the 30S and 50s ribosomal subunit for *Thermusthermophiles* (Schluenzen et.al 2000, Carter et.al 2000), *Haloarculamarismortui (Ban et.al 2000)* and *Deinococcusradiodu-rans* (Harmset.al 2001) have significantly extended the knowledge base of RNA three-dimensional structure. However, the knowledge of three-dimensional space by these techniques is still limited. Thus, computational methods such as comparative and *de novo* approaches and molecular dynamic simulations are intensively used as alternative tools to predict the three-dimensional structures and dynamic behaviour of proteins (Hsuan et.al 2005). Homology modeling often provides a useful to predict 3-D model for a protein that is related to at least one known protein structure based on protein sequence and alignment to one or more proteins of known structure (Marti-Renom et al., 2000; Fiser, 2004; Misuraand Baker, 2005; Petrey and Honig, 2005; Misura et al., 2006). Structural Dynamics of the *E. col*i 70s ribosomal proteins S13, S19, L2, L5, and L14 are seen to be involved in the control of the relative movement of the subunits through match, and their inter-subunit bridges (Frank et.al 2003). The gene sequences encoding ribosomal proteins L11 and L13 were characterized and their expression was analysed during development under cadmium stress (Planello et.al 2007). The structure of the *Escherichia coli* ribosomal protein L25 has been determined by using NMR studies (Matthias et.al 1998). The nature of post translational modifications of the ribosomal proteins of *E. coli* has been determined (Nesterchuk 2011). MALDI-MS has been successfully applied for identifying and characterizing multi protein complexes. Two important mass spectrometry methods used in identifying protein components of macromolecular complexes are the frequently applied "bottom-up" and the less widely used "top-down" strategies. The bottom-up strategy involves enzymatic digestion of intact proteins to generate peptides that are analysed by the mass spectrometer (Geng et.al 2000, Rid et al 2002, Aebersold et.al 2003, Delahunty et.al 2007). L31 is a component of a bacterial ribosome that has not been studied adequately. In the present study, we analysed expression and homology modelling of distinct ribosomal protein L31 of E.coli Nissle 1917 under Mercury stress was performed to predict the three dimensional structure.

MATERIAL AND METHODS

Collection of animals

For the present work, male and female healthy adult animals (6±1 cmcarapace width) were used. Healthy adult crabs (5.5 cm mean carapace width,105 gms wt. females and 6 cm mean carapace width 120 gms wt. males) were purchased from regular animal supplier kept in the laboratory in disinfected plastic tubs, the water in the tubs was changed every day and fed with minced chicken. The crabs were acclimatized in the laboratory for 7 days. Barytelphusa Guerini crabs were collected from local market, Hyderabad.

Collection of Hemolymph:

Hemolymph was collected from unsclerotized membrane from the ventral side with Insulin syringe and each crab was subjected to a single bleed amounting to 1–2 ml of Hemolymph at different time intervals 2h, 6h, 12h, 24h and 48 hrs. The collected hemolymph was immediately diluted with 1:1 ice cold anticoagulant solution for further biochemical studies.

Two dimensional polyacrylamide gel electrophoresis (2D- PAGE)

2D PAGE was carried out to study the differential expression of proteins. Isoelectric focusing (IEF) was performed using 7 cm IPG strips of pH range 3–10 using the BioRad IEF cell. IPG strips were rehydrated passively with ReadyPrep2DRehydration Buffer (Bio- Rad cat. no. 163-2083) premixed with the sample (100_g/strip). IEF was performed in IEF cell using 7 cm focussing tray, the starting voltage was from 0V and the end voltage was 4000Vwith total 10,000Vh at 20^{o}C rest of the procedure was followed as per Bio-Rad instruction manual no. 163-2099. After IEF, proteins were separated in the second dimension by using 12% SDS-PAGE gel and 80V was maintained throughout while running the second dimension. Gels were stained using sypro ruby. Images were acquired using Fluor-S-Multi imager (Bio-Rad) using visible light source, and differentially expressed proteins were detected manually and treated as separate spots.

MALDI-TOF Mass Spectrometry and Peptide Mass Fingerprinting (PMF) Analysis

Peptide mixture was mixed with an equal volume of matrix solution (10 mg/ml -cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile containing 0.1% TFA) and the mixture was analyzed on MALDI-TOF MS (Model Voyager-DE STR, Applied Biosystems, Foster, CA, USA). The spectra measured for unknown peptides were compared against the mass peaks derived from calibration standards. Spectra were collected over the mass range of 800–3500 Da and integrated with the MASCOT 2.2 search engine (Matrix Science, http://www.matrixscience.com/) was used for spot proteins identification by querying the trypsin digested peptide fragment data using the reference database NCBInr. Known keratin masses and trypsin autodigest products were excluded from the searches. Protein homology was assigned, if at least four peptide masses were matching within a maximum of 100-ppm error spread across the data set. The number of missed cleavage sites was allowed up to 1. Search result scores which is greater than 48 was considered to be of significant difference ($p<0.05$).

Homology Modelling

Identification of domain and template for 50s Ribosomal protein L31

The sequence of 50s Ribosomal protein L31 was obtained from MS data of Barytelphusa Guerini. The sequence from Barytelphusa Guerini was submitted to SBASE server for domain prediction. The domain was submitted to Interproscan to identify the function and was searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) (Altschul 1990; McGinnis S., & Madden T.L., 2004) program against PDB (Protein Databank). Sequence that showed maximum identity with high score and less E-value was used as a reference structure to build a three dimensional model for 50s Ribosomal protein L31.

Alignment

The template sequence collected from Uniprot database was aligned with 50S ribosomal protein L31 using CLUSTALX software. The structurally conserved regions (SCRs) of both protein and template were determined by multiple sequence alignment (Needleman and Wunsch 1970).

3D Model building

The initial model of 50S ribosomal protein L31 was built by using homology-modeling methods and the MODELLER9V7 software. It is a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain Cα- Cα distances, main-chain N-O distances, main-chain and side-chain dihedral angles.

Variable regions (VRs) are defined as the sections of the proteins that exist between the SCRs in the sequence. To construct the structural variable regions, a loop-searching algorithm over the data bank of known crystal structure was used. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms (Sali, A. and Blundell, T. L. 1993).

Molecular Dynamics studies of 50s Ribosomal protein L31

The initial model was improved by Energy Minimization. After 1000 steps of Conjugate Gradient (CG) minimization performed, Molecular Dynamics (MD) simulation was carried out to examine the quality of the model structures by checking their stability via performing 500 ps simulations under 1 atm constant pressure and 310 K constant temperature (NPT). An explicit solvent model TIP3P water was used (Jorgensen et al 1983). The homology model was constructed with a 10 A^0 water cap from the centre of mass of Ribosomal protein L31. No periodic boundary conditions were included in this study. A cutoff of 12 A^0 (switching function starting at 10 A^0) for van der Waals interactions was assumed. An integration time step of 2 fs was used, permitting a multiple time-stepping algorithm (Grubmuller 1991) to be employed in which interactions involving covalent bonds were computed every one time step and short-range non bonded interactions were also computed in every two time steps, and long-range electrostatic forces were computed every four time steps. The pair list of the non bonded interaction was recalculated in every ten time steps with a pair list distance of 13.5 A^0. The short-range non bonded interactions were defined as van der Waals and electrostatic interactions between particles within 12 A^0. A smoothing function was employed for the van der Waals interactions at a distance of 10 A^0. All calculations mentioned above used the NAMD 2.5 software (Kale et al 1999) using CHARMM27 force field for lipids and proteins. CHARMM27 force-field parameters were used in all simulations in this study. Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved.

Structure Validation

After the optimization procedure, the final structure obtained was checked by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) (Brunger 1992) and environment profile using ERRAT graph (Structure Evaluation server) (Laskowski *et al* 1993).

Docking studies

Docking of 50S ribosomal protein L31 with Sortase A was carried out using GOLD (Genetic Optimization of Ligand Docking) software which was based on genetic algorithm (GA). This process is based on protein partial flexibility and ligand full flexibility. In this work, 50S ribosomal protein L31 was docked to Sortase A active site. The binding of ABC Transporter1 to the amino acids in the active site were studied using molecular mechanics method. The options for GA run were taken such as population size (100), pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator options for crossover, mutation, and migration were set to 100, 100 and 10 respectively. Default parameters of 6.0 A° for van der Waals forces and 3.0 A° (dH-X) for hydrogen bonds were selected. During docking, the default algorithm speed was selected and the compound binding site in the Sortase A was defined within a 10 A° radius with the centroid as CE atom of THR247. The number of poses for compound was set 10, and termination was assigned if the top three bound conformations of Sortase A were within 1.5A° RMSD. After docking, the individual binding poses of Sortase A were studied and also their interactions. The best and most favorable conformation of Sortase A was selected.

Gold Score fitness

Gold Score performs a force field based scoring function and was made up of four components: 1. Protein-ligand bonding (external H-bond); 2. Protein-ligand van der Waals energy (external vdw); 3. ligand internal van der Waals energy (internal vdw); 4. ligand intramolecular hydrogen bonding (internal- H- bond). The external vdw score was multiplied with 1.375 factor when the total fitness score was calculated. This was an correction to develop protein-ligand hydrophobic interaction. The fitness function has been minimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

RESULTS AND DISCUSSION

MALDI-TOF RESULTS

In this study we examined haemolymph of Barytelphusa Guerini before and after the exposure to staphylococcus aureus to identify differentially expressed proteins. Cells were treated with 0.02 mM mercury, by using 2D-PAGE and MALDI- TOF- Ms. Proteome analysis can deliver more accurate and comprehensive information than genomic/ transcriptomic, because protein expressions are regulated not only at transcriptional but also at translational levels (Humphery-Smith et al., 1997). After the treatment of Barytelphusa Guerini with staphylococcus aureus many proteins were up or down regulated, these proteins are called stress proteins. Mercury-responsive proteins were sorted into different functional classes: induction of heat shock proteins, Uncharacterized HTH-type transcriptional regulator, induction proteins involved in lipoic acids biosynthesis, and basic ribosomal proteins, The criterion for conformation of 50S ribosomal protein L31 involved enzymatic digestion of differentially expressed proteins to generate peptides masses that are analyzed by using Mascot database using the reference database NCBInr, and considered higher average sequence coverage per ribosomal protein and overall number of peptides identified. We identified 50S ribosomal protein L31 mass of 7866Da with 42% sequence similarities (Figure I).

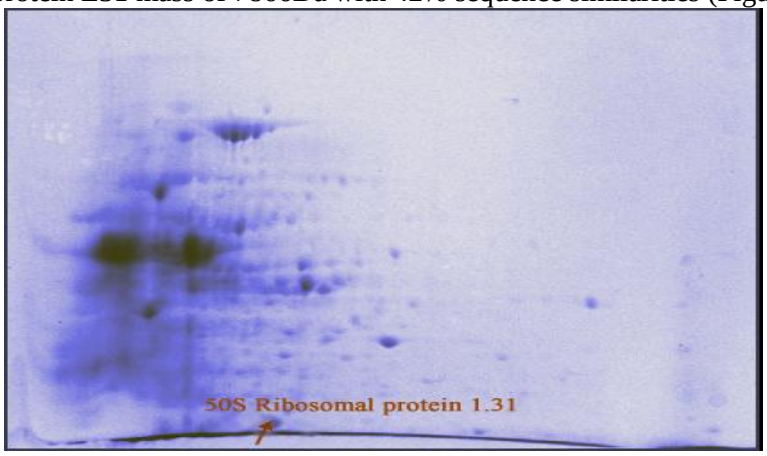


Figure I: Identification of Ribosomal L31 through Mass spectrometry

50S ribosomal protein L31 make up a great part of the proteome in the pH range of 6–11. Two of these ribosomal proteins were up regulated at high Hg (II) load, indicating a generally enhanced protein synthesis under the stress conditions caused by high Hg (II) load, ribosomal proteins are most abundant proteins in bacterial cells during the exponential phase and ribosome is place assembling proteins, play essential role in metabolism.

HOMOLOGY MODELLING

50S ribosomal protein L31 function was identified using Interproscan (Fig. II). In our study, the 3D model is made up of residues (1-87) because the active domain region was identified in between these residues by the SBASE server (Rosenberg *et al* 2005) (Fig. III).

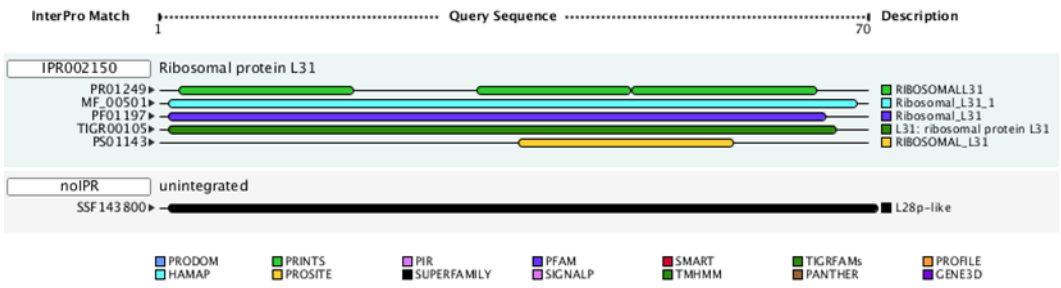


Figure II: Identification of Function the protein

Figure III: Domain identification of 50s Ribosomal L31

In the results of BLAST search against PDB, only one-reference protein, including 2AW4 (Chain A; Crystal structure of the bacterial ribosome from Escherichia coli at 3.5 A resolution) (Schuwirth et al 2005) with Ribosomal L31 have a high level of sequence identity and the identity of the reference protein with the P-glycoprotein domain is 40%. Structurally conserved regions (SCRs) for the model and the template were determined by multiple sequence alignment (Fig. IV). Prevoius models were generated using different templates (Gao et al 2003, Tung et al 2002). More recently, Tung *et al*, analyzed the structure of 30s Ribosomal subunit of E.coli.

```
CLUSTAL 2.0.11 multiple sequence alignment

ribosomal       MKPNIHPEYRTVVFHDTSVDEYFKIGSTIKTDREIELDGVTYPYVTIDVSSKSHPFYTGK
2AW4            MKKDIHPKYEEITAS-CSCGNVMKIRSTVGHD-----------LNLDVCSKCHPFFTGK
                ** :***:*. :.    * .: :** **:  *            :.:**.**.***:***

ribosomal       LRTVASEGNVARFTQRFGRFVSTKKGA
2AW4            QRDVATGGRVDRFNKRFN--IPGSK--
                 * **: *.* **.:**.  :. .*
```

Figure IV: sequence alignment between ribosomal L31 protein and template (2AW4)

3.1 Homology Modelling of 50S ribosomal protein L31 Domain

The options used for running the Modeller9v7 are tabulated (Table I). The model generated showed three helices and was improved by molecular dynamics method and the final stable structure was further checked by VERIFY-3D graph (Fig.V).

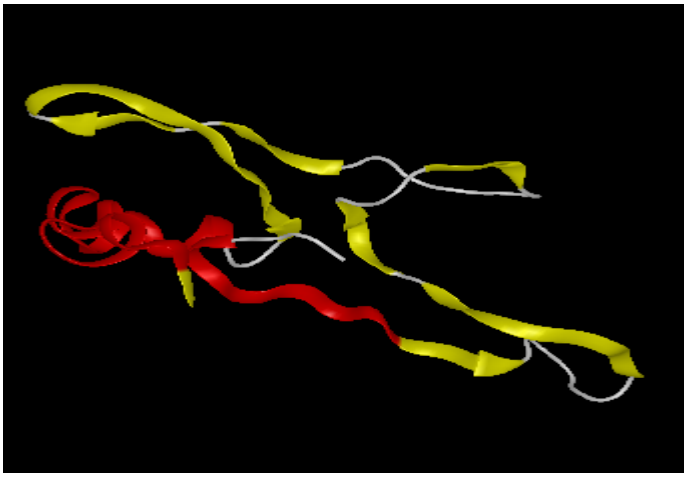


Figure V: Three dimensional structure of Modelled Ribosomal L31 protein

Tab. I: Options used in MODELLER program

INCLUDE	#include the predefined TOP routines
SET ALNFILE	= ‘alignment.ali’
SET KNOWNS	= ‘2AW4’
SET SEQUENCE	= ‘Ribosomal’
SET HETATM_IO	= on
SET WATR_IO	= off
SET HYDROGEN	= off
SET STARTING_MODEL	= 1
SET ENDING_MODEL	= 50
CALL ROUTINE	= ‘model’

3.2 Validation of 50S ribosomal protein L31 Domain

The position of amino acids in the 50S ribosomal protein L31 was computed with the PROCHECK program. After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program (Fig.VI). The φ and ψ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized (Table II).

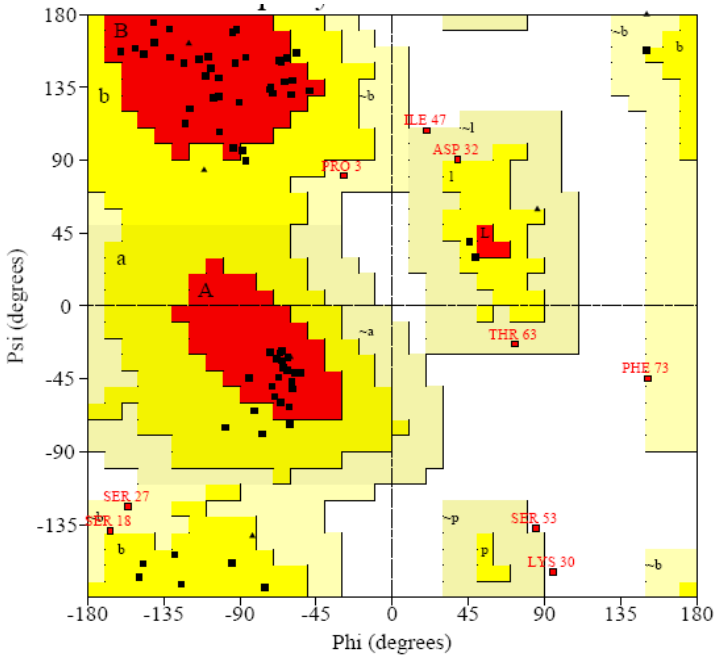


Figure VI: Ramachandran's map of Ribosomal L31 protein

Tab. II: % of residue falling in the core region of the Ramachandran's plot

Residues in most favoured regions	53	70.7%
Residues in additional allowed regions	14	18.7%
Residues in generously allowed regions	6	8.0%
Residues in disallowed regions	2	2.7%
---- ------		
Number of non-glycine and non-proline residues	75	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	6	
Number of proline residues	4	

Total number of residues	87	

Altogether 99.5% of the residues of 50S ribosomal protein L31 was in favoured and allowed regions. The compatibility score above zero in the VERIFY-3D program showed except four, all residues are reasonable which makes us to believe that the structure of the 50S ribosomal protein L31 is reliable (Fig. VII).

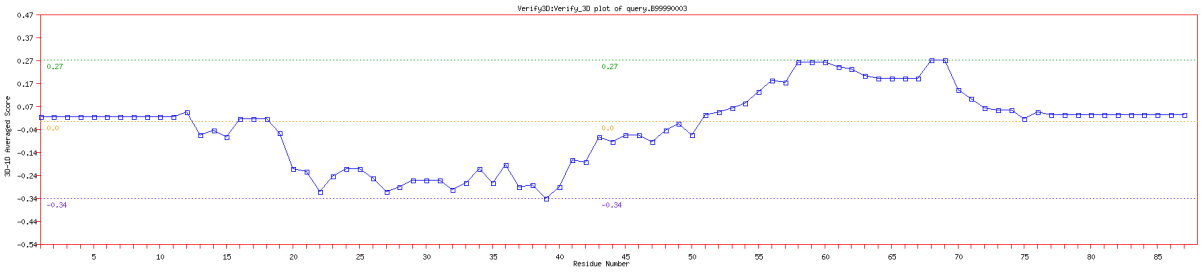


Figure VII: The 3D profiles verified results of P-glycoprotein model

The overall PROCHECK G-factor of 50S ribosomal protein L31 was -2.32 and VERIFY 3D environment profile was good. The weighted root mean square deviation of Cα trace between 2AW4 and 50s Ribosomal subunit was identified by superimposition of Cα trace and the calculated value was 1.88 A^o (Fig. VIII and IX).

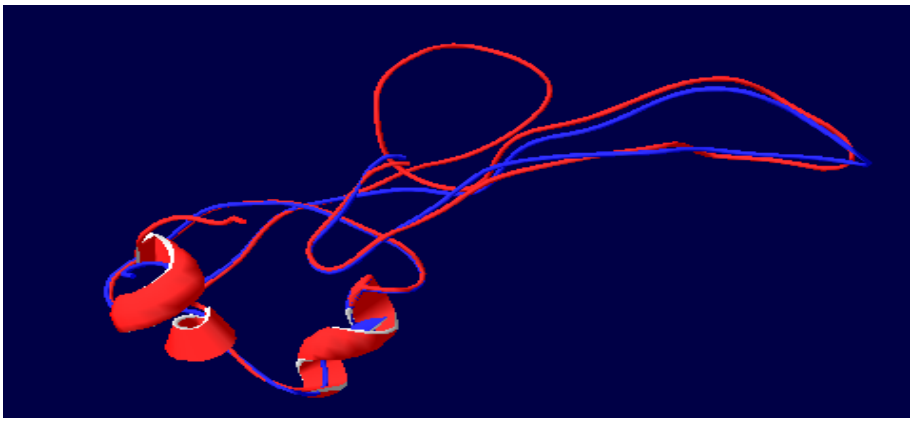
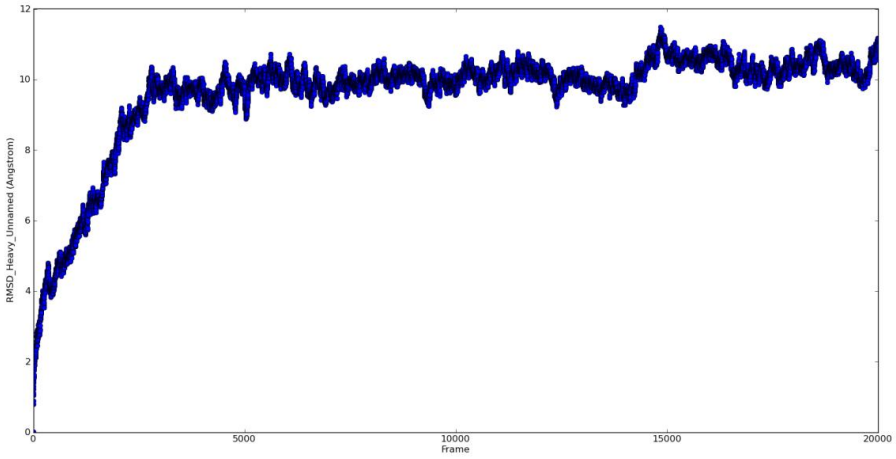


Figure VIII: Superimposition between ribosomal L31 (Red colour) and 3AW4 (Blue colour)

Figure IX: Calculated RMSD graph of molecular dynamics simulations of Ribosomal L31 protein using NAMD software. Time (Ps) was taken in X-axis and RMSD in Y-axis.



Docking studies of 50S ribosomal protein L31

Studies of **50S ribosomal protein L31** was performed with Sortase A, a membrane protein of *S.aureus.* The structure of this was constructed and optimized using SPDBV software. The selected docked conformations of **50S ribosomal protein L31** into the Sortase A binding site are shown in Fig.X. The docked results (Table 3) revealed that the **50S ribosomal protein L31** the inhibitory peptide of Sortase A of *S.aureus.*

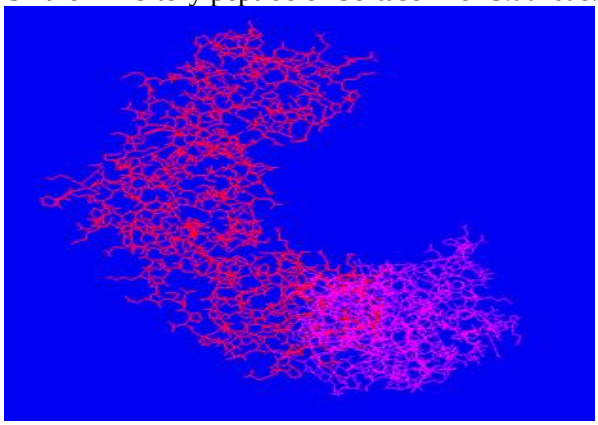


Fig X: Docking results of 50S ribosomal protein L31 with the active site of Sortase A.

Table 3: Docking results

Docking Score (K.Cal/mol)	Protein
-54.21	**Ribosomal L31**

CONCLUSION

In this work we have identified antimicrobial peptides from Barytelphusa Guerini after inoculating the staphylococcus aureus. The haemolymph was subjected to MALDI. From the MALDI-MS data we identified the protein as 50s Ribosomal L31. After the protein sequence identification, it was submitted to interproscan for functional identification. A three dimensional structure was identified and molecular dynamics studies was performed. From the structure validation studies it was shown that our generated model for 50s Ribosomal L31 protein is reliable. This structure was docked to the active site of Sortase A from staphylococcus aureus. From the docking analysis it was identified that 50s Ribosomal L31 inhibiting the Sortase A of Staphylococcus aureus.

REFERENCES

Aebersold R, Mann M. (2003). Mass spectrometry-based proteomics. Nature 422:198–207.

Andrew P. Carter, William M. Clemons, Ditlev E. Brodersen, Robert J. Morgan-Warren, Brian T. Wimberly& V. Ramakrishnan. (2000). Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. Nature 407, 340-348

Chen, F.W., Ioannou, Y.A., (1999). Ribosomal proteins in cell proliferation and apoptosis. Int. Rev. Immunol. 18, 429–448.

Delahunty CM, Yates JR 3rd. (2007). MudPIT: multidimensional protein identification technology. Biotechniques 43:563, 565, 567.

Eric R. Dabbs. (1981). The Gene for Ribosomal Protein L31, rpmE, Is Located at 88.5 Minutes on the Escherichia coli Chromosomal Linkage Map. .Journal Of Bacteriology, Oct. P. 379-382.

Geng M, Ji J, Regnier FE. (2000). Signature-peptide approach to detecting proteins in complex mixtures.J. Chromatogr. A 870:295–313.

HaixiaoGao, JayatiSengupta, Mikel Valle, Andrei Korostelev, NarayananEswar, Scott M. Stagg, Patrick Van Roey, Rajendra K. Agrawal, Stephen C. Harvey, Andrej Sali, Michael S. Chapman,Joachim Frank., (2003). Study of the Structural Dynamics of the *E. coli* 70S Ribosome Using Real-Space Refinement. Cell, Vol. 113, 789–801, June 13.

Hsuan-Liang Liu1 and Jyh-Ping Hsu (2005). Recent developments in structural proteomics for protein structure determination Proteomics, 5, 2056–2068.

J.L. Martínez-Guitarte, R. Planelló, G. Morcillo., (2007). Characterization and expression during development and under environmental stress of the genes encoding ribosomal proteins L11 and L13 in Chironomusriparius .,Comparative Biochemistry and Physiology, Part B 147 590–596.

JoergHarms, FrankSchluenzen, RazZarivach, AnatBashan, SharonGat, IlanaAgmon, Heike Bartels, François Franceschi and Ada Yonath. (2001). High Resolution Structure of the Large Ribosomal Subunit from a Mesophilic Eubacterium. cell 107, 679-688

Liwei Sun, KanaeTeramoto, Hiroaki Sato, Masaki Torimura., (2006). Characterization of ribosomal proteins as biomarkers for matrix-assisted laser desorption/ionization mass spectral identification of *Lactobacillus plantarum*.,Rapid communication in mass spectroscopy vol 20 issue 4pages 3789 -3798.

M. V. Nesterchuk, P. V. Sergiev, O. A. Dontsova., (2011). Posttranslational Modifications of Ribosomal Proteins in *Escherichia coli* VOL. 3 № 2 (9) Actanaturae 23-33.

Matthias Stoldt, Jens Wo¨ hnert,Matthias Go¨ rlach and Larry R.Brown. (1998). The NMR structure of Escherichia coli ribosomal protein L25 shows homology to general stress proteins and glutaminyl-tRNA synthetases. The EMBO Journal Vol.17 No.21 pp.6377–6384

Mayer, C., Bierhoff, H., Grunmt, I., (2005). The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. Genes Dev. 19, 933–941.

Nenad Ban, PoulNissen, Jeffrey Hansen, Peter B. Moore, and Thomas A. Steitz. (2000). The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution .sciences 289.,905-920.

Olson, M.O., (2004). Sensing cellular stress: another new function for the nucleolus? Sci. STKE 224, 1–10.

passim Draper, D.E., Reynaldo, L.P., (1999). RNA binding strategies of ribosomal proteins. Nucleic Acid Res. 27, 381–387.

Ramakrishnan V and White SW (1998). Ribosomal protein structures: insights into the architecture, machinery and evolution of the ribosome. Trends in Biochemical Sciences 23: 208–212.

Reid GE, McLuckey SA. (2002). 'Top down' protein characterization via tandem mass spectrometry. *J. Mass Spectrom.* 37:663–75.

SchluenzenF,TocilJ.A.Zanivach.R (2000). Structure of a functionally activated small ribosomal subunit at 3.3, Angstroms resolution cell 102:615-623.

Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M., Cate, J.H. (2005). Structures of the bacterial ribosome at 3.5 A resolution Journal: Science 310: 827-834.

Wimberly,B.T.Brodersen, D.E.Clemenson,W.M, Jr., Morgon-Worrren,R.J.,Carter,A.S vonrhein.C.,Hartsch.T., and Ramakrishnan V., (2000). Structure of 30s Ribosomal subunit .Nature 407:327-339.

Wittmann, H. G. (1974). Two-dimensional polyacrylamide gel electrophoresis for separation of ribosomal proteins. Methods Enzymol. 30:497–505.

Wittmann, H. G., G. Stofflet, I. Hindennach, C. G. Kurland, E. A. Birge, L. Randall-Hazelbauer, M. Nomura, E. Kaltschmidt, S. Mizushima, R. R. Traut, and T. A. Bickle. (1971). Correlation of 30S ribosomal proteins of *Escherichia coli* isolated in different laboratories. Mol. Gen. Genet. 111:327–333.

Wittmann, H. G., J. A. Littlechild, and B. Wittmann-Liebold. (1980). Structure of ribosomal proteins, p. 51–88. *In* G. Chambliss, G. R. Craven, J. Davies, K. Davies, L. Kahan, and M. Nomura (ed.), Ribosomes: structure, function and genetics. University Park Press, Baltimore, Md.

Wittmann-Liebold B (1986). Ribosomal proteins: their structure and evolution. In: Hardesty B and Kramer G (eds) Structure, Function and Genetics of Ribosomes. New York: Springer-Verlag.

Humphery-Smith, I., Cordwell, S. J., & Black stock, W. P., (1997). Proteome research: Complementarity and limitations with respect to the RNA and DNA worlds. Electrophoresis, *18*, 1217-1242.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com