ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Membrane Drug Target Identification in Mycoplasma Pneumonia- A Subtractive Genomic Approach.

A Ranganadha Reddy[1], K Praveen Kumar[2], M Indira[1], TC Venkateswarulu[1], D Ramya Krishna[1], and S Karisma[1], and K Supriya[1].

[1]Department of Biotechnology VFSTR University Guntur Andhrapradesh 522 213, India.
[2]Department of Information Technology VFSTR University Guntur Andhrapradesh 522 213, India.

ABSTRACT

The ease of use of genome sequences of pathogens has provided a remarkable quantity of information that can be used in identification of drug targets and vaccine production. The application of in silico subtractive genomics technology drastically shortens the time required for such purposes. Subtractive genomics approach enables the subtraction dataset between the host and pathogen. The aim of present work is to identify the Drug targets in Mycoplasma Pneumonia by subtractive genomic approach which helps to identify the homolog's and Non homologs between the host and pathogen. The availability of complete Proteome sequence information of Mycoplasma Pneumonia from NCBI-FTP site helps to perform the BLAST against human proteome database. The screening of these proteins using different database like essential genes (DEG), metabolic pathways KEGG helps to identify essential proteins which may be used as drug targets against Mycoplasma pneumonia. Prediction of these essential proteins of the outer membrane of the pathogen also could be potential vaccine targets

Key words: Pneumonia, Subtractive genomic approach, ICM molsoft, NCBI-FTP

Corresponding author

ISSN: 0975-8585

INTRODUCTION

There are a lot of ways to detect potential drug target such as uncharacterized vital genes, virulence genes, species–specific gene and membrane transporter etc. [1] Comparative genomics provides a new approach to identify new drug targets amongst earlier known targets based on their related biological function in pathogen and host. In the planned work subtractive genomic approach is used, where subtraction dataset comparing two genomes i.e. pathogen and host. This approach is effectively used in quite a few other bacteria such as Pseudomonas aeruginosa[2], Helicobacter pylori[3] etc. The attempt has been made to find the least number of genes necessary for a self-replicating cell, since the whole genome of Mycoplasma has been sequenced. A smallest gene set necessary for a species, which could be deduced from conserved genes in the analyzed genome.[4] "The least possible group of genes that would be adequate to retain a functioning cellular life form under the most favorable conditions possible, i.e. in the presence of full complement of essential nutrients and in the absence of environmental stress" is defined as minimal gene set or vital genes.[5][6][7] In *Mycoplasma genitalium* 265-350 protein coding genes are identified as vital under laboratory growth condition, which is orthologous to the *Mycoplasma pneumonia*.[8] In the subsequent work subtractive genomics and Database of Essential Gene (DEG) is used to analyze the genes of *Mycoplasma pneumoniae* for finding potential target at the outer surface of pathogen, might be used as drug target. *Mycoplasma pneumoniae* is a potential bacterial pathogen which lacks cell wall and surrounded by a cytoplasmic membrane. It causes atypical pneumonia in human *Mycoplasma pneumonia* is transmitted from person-to-person contact through respiratory secretions during coughing and sneezing. The incubation period is usually 14-21 days. The whole genome of *Mycoplasma pneumoniae* has been sequenced. The M129 strain of *Mycoplasma pneumoniae* is linear single stranded of length 816,394 base pairs with an average G+C contain of 40.0 mol %.[9] All the major classes of cellular process and metabolic pathway are briefly described. A number of activities/functions present in *Mycoplasma pneumoniae* according to experimental substantiation, but genes or proteins involved in motility, chemotaxis and management of oxidative stress are not known still. [10]

MATERIAL AND METHODS

DATABASES

Database: A database is an assemblage of information that is structured so that it can easily be accessed, managed, and updated. [11]

Data mining or discovery: Data mining or knowledge discovery is the computer-oriented process of digging and analyzing large volumes of data and finally extracting the importance of the data. Data mining can be performed on data represented in quantitative, textual, or multimedia forms. [12]

NCBI (National Center for biotechnology information): The National Center for Biotechnology Information (NCBI) [18][13] is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine.

Swiss-Prot: Swiss-Prot was created in 1986 by Amos Bairoch and developed by the Swiss Institute of Bioinformatics and subsequently developed by Rolf Apweiler at the European Bioinformatics Institute.[13][14] Swiss-Prot aimed to provide reliable protein sequences associated with a high level of annotation a minimal level of redundancy and high level of integration with other databases.

Protein Data Bank: PDB consists of 3D (Three Dimensional) data of experimentally determined structures of proteins and nucleic acids[14][15] established at Brookhaven National Laboratory.[15][16] The archive is managed by the Worldwide Protein Data Bank organization (wwPDB), whose mission is to ensure that a single, global PDB data archive is and will remain freely and publicly available.[16][17]

NCBI (National Center for biotechnology information): The National Center for Biotechnology Information (NCBI) [18] is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of

Health. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine.

DEG (Database of Essential Genes): Database of Essential Genes (DEG) that contains all the essential genes currently available. These genes include the essential genes identified in the genomes of *M.genitalium* , *H.influenzae* , *V.cholerae* , *S.aureus* , *E. coli* and *Saccharomyces cerevisiae*., in which the essential genes are collected from a large number of related references. The essential genes in yeast genome were extracted from the yeast genome database which is maintained by the Munich Information Center for Protein Sequences.

TOOLS

Basic Local Alignment Search Tool **(BLAST)**: Nowadays Similarity searching, including sequence comparison, is one of the principal techniques used by computational biologists and has found widespread use among biologists in general. The most popular tool for this purpose is BLAST [17][18] (Basic Local Alignment Search Tool) which performs comparisons between pairs of sequences, searching for regions of local similarity. NCBI BLAST is available from the NCBI [18][13] (National Center for Biotechnology Information).

MOLSOFT: ICM MOLSOFT algorithm was adopted for comparative modeling which provides an accurate and efficient module to build loops and side chains found non-identical in sequence. ICM mol soft algorithm contains robust modeling tools and high levels of accuracy with fast model building.[19]

Real Space Automated Conformer Generation (RAPPER): RAPPER [20][21] is an ab initio conformational search algorithm for restraint-based protein modeling. It has been used for all-atom loop modeling, whole protein modeling under limited restraints comparative modeling, ab initio structure prediction, structure validation and experimental structure determination with X-ray and nuclear magnetic resonance spectroscopy. Web interfaces are available on this website for Ramachandran plot analysis.

METHODOLOGY

RETRIEVAL OF TARGET SEQUENCE:

The protein sequence of the protein Dihydroxy acetone kinase system protein of mycoplasma pneumoniae had been retrieved from UniProt, and saved in fasta format that gives the specific information regarding the number of amino acids in the sequence and other sequence related information

PERFORMING TEMPLATE SEARCH:

The protein sequence of the protein Dihydroxy acetone kinase system protein uncharacterized had been retrieved from UniProt and the search for the template had been done using Blast algoritham.Template selection by blast searching Algorithm

HOMOLOGY MODELING USING MOLSOFT:

The query is loaded by going to windows and clicking on sequence editor. Single letter residues are then displayed. Color residues are displayed next.

PDB search is done by going to homology and giving the query. First template in the result is loaded. Then the two sequences are and aligned by freeze in align of homology. The model is build by going to homology and selecting homology model.

Homology modeling

The steps to creating a homology model are as follows:

- Identify homologous proteins and determine the extent of their sequence similarity with one another and the unknown.

ISSN: 0975-8585

- Align the sequences.
- Identify structurally conserved and structurally variable regions
- Generate coordinates for core (structurally conserved) residues of the unknown structure from those of the known structure(s).
- Generate conformations for the loops (structurally variable) in the unknown structure.
- Build the side-chain conformations.
- Refine and evaluate the unknown structure.

FLOW CHART OF HOMOLOGY MODELING

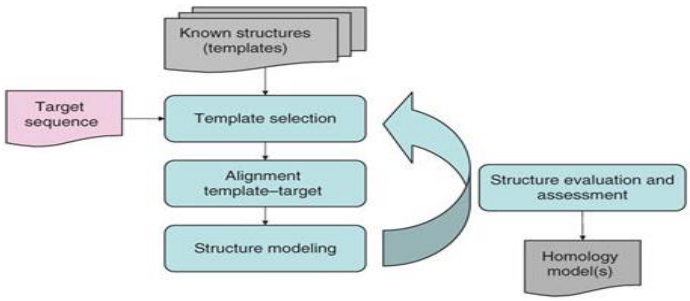


Figure-1: The four main steps of homology modeling of protein i.e. template selection, target–template alignment, model building and model quality evaluation [22]

VALIDATION OF MODELED PROTEIN USING RAPPER:

As the loop of the structure is built up and the terminals are removed, the modeled structure is now given for validation through RAPPER. The structure is given in .pdb form to it to analyze the structure and to generate the RAMACHANDRAN plot of the structure to find the validity of the structure. This is again also carried out after minimization of the structure is done

RESULTS AND DISCUSSIONS

Retrival of protein sequence from Swissprot :

The details of the Dihydroxy acetone kinase system protein like amino acid lengths of N-and C-terminus, helix lengths etc were obtained from Swiss-Prot database with accession number **P75231.** The details of sequence annotation are shown below.

Blast search against Human Proteome Database:

```
>sp|P75231|Y547_MYCPN Uncharacterized protein MG369 homolog OS=Mycoplasma
pneumoniae (strain ATCC 29342 / M129) GN=MPN_547 PE=3 SV=1
MSTVNLSNFIDMLRLGCKNIANNFEYINQLNVFPVPDGDTGTNMKVTLSEAFKKLESEIS
HIKSFSDLGKSFTRDLLLFSRGNSGVIFSQIMKGFFSDMISTKTATETELGIEDFATAFI
KAEEVAYKNVSKPVEGTMLTVIRLISTDFKNQKNRAKTVQKLFEQVIKTAWQTVKKTPQM
LPVLKASGVVDSGAYGFACFLEGMLSFYGEKATLNDGKLTSAELSQMTISGEKHVTEEEF
GYCTEYVLKLGMSVSQEVEKQKFNQKKFESKVSKIATSVVVASDKDNGFVKVHAHTEKPN
LLLELGLNYGEFELVKIENMNLQVAKQKPAPVKRNIKPAIVVTVPTEAFADRIREDYDIQ
```

ISSN: 0975-8585

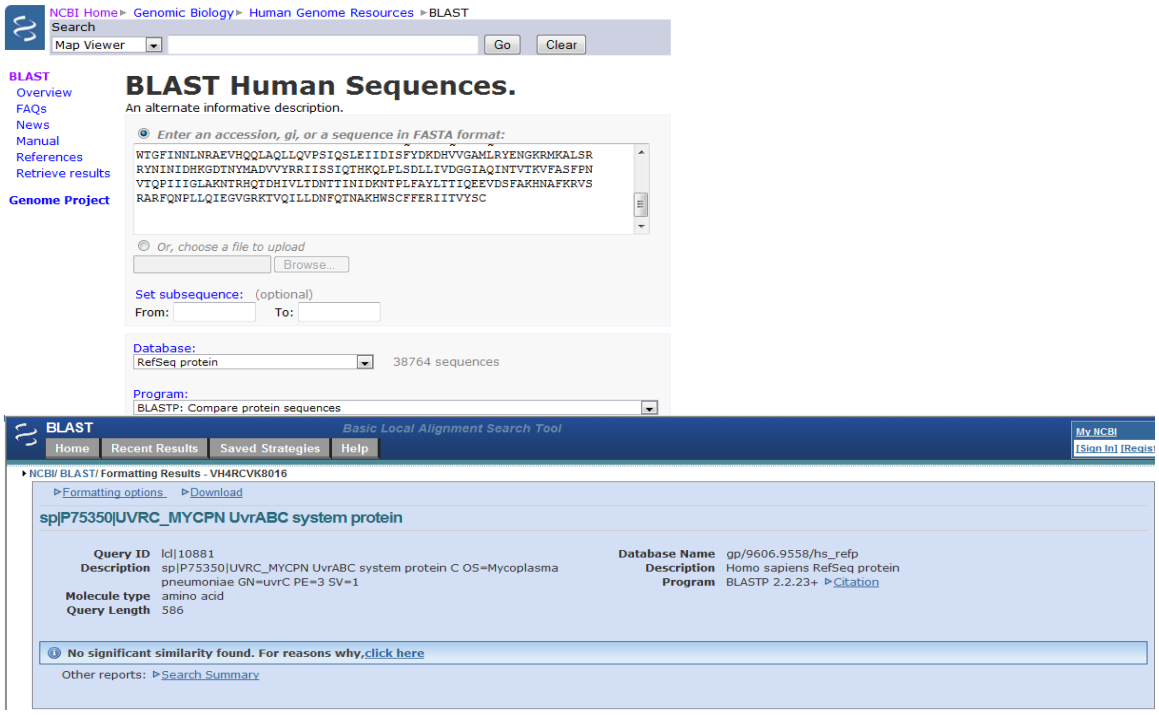


Figure-2: Shows Dihydro acetone kinase protein of Mycoplasma Pneumoniae is non homologous to Human proteome database.

Database of essential Genes Results:

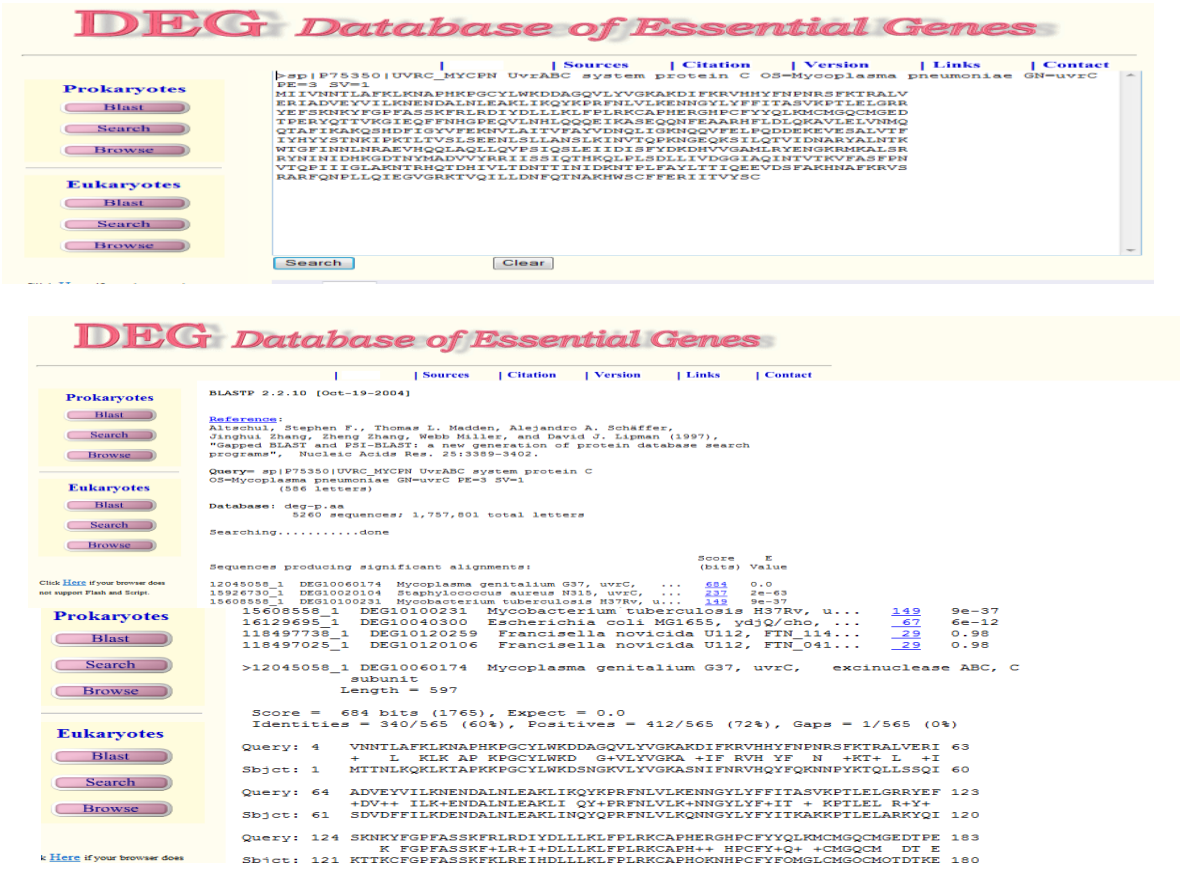
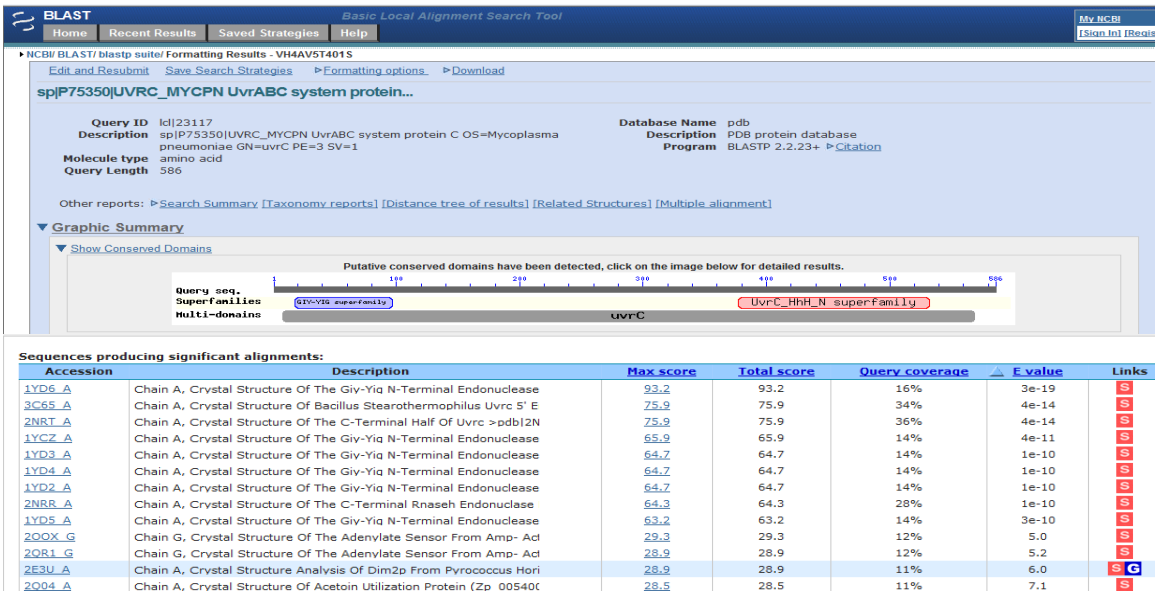


Figure-3: Shows Database of essential genes results which indicate Dihydro acetone kinase protein is essential for Mycoplasma Pneumonia

ISSN: 0975-8585

Template selection by blast searching Algorithm:



Accession	Description	Max score	Total score	Query coverage	E value	Links
1YD6_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	93.2	93.2	16%	3e-19	S
3C65_A	Chain A, Crystal Structure Of Bacillus Stearothermophilus Uvrc 5' E	75.9	75.9	34%	4e-14	S
2NRT_A	Chain A, Crystal Structure Of The C-Terminal Half Of Uvrc >pdb\2N	75.9	75.9	36%	4e-14	S
1YCZ_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	65.9	65.9	14%	4e-11	S
1YD3_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	64.7	64.7	14%	1e-10	S
1YD4_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	64.7	64.7	14%	1e-10	S
1YD2_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	64.7	64.7	14%	1e-10	S
2NRR_A	Chain A, Crystal Structure Of The C-Terminal Rnaseh Endonuclase	64.3	64.3	28%	1e-10	S
1YD5_A	Chain A, Crystal Structure Of The Giy-Yig N-Terminal Endonuclease	63.2	63.2	14%	3e-10	S
2OOX_G	Chain G, Crystal Structure Of The Adenylate Sensor From Amp- Acl	29.3	29.3	12%	5.0	S
2OR1_G	Chain G, Crystal Structure Of The Adenylate Sensor From Amp- Acl	28.9	28.9	12%	5.2	S
2E3U_A	Chain A, Crystal Structure Analysis Of Dim2p From Pyrococcus Hori	28.9	28.9	11%	6.0	S G
2Q04_A	Chain A, Crystal Structure Of Acetoin Utilization Protein (Zp_00540(	28.5	28.5	11%	7.1	S

Figure-4 : BLAST result for Dihydro acetone kinase protein

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between protein or nucleotide sequences. The program compares nucleotide or protein sequences to sequence in a database and calculates the statistical significance of the matches. The target sequence i.e., Dihydro acetone kinase protein (UniProt ID: P75231) was searched against the protein database by using BLAST tool. From the BLAST results as shown in Figure-2, Figure-3 and Figure-4. we observed that four proteins (PDB IDs: 1BLS-A, 3C65-A, 2NRT-A, 1YCZ-A) are showing the maximum identity with the target sequence. Among the four proteins obtained, 1BLS-A selected for further proceedings.

Three-Dimensional Structure Prediction of Dihydroxy acetone kinase Protein by MOLSOFT Software: Three dimensitional structure of Dihydroxy acetone kinase protein (UniProt ID: P75231) as shown in **Figure-5 and Figure-6** was predicted by using the tool MOLSOFT ICM by taking **1BLS-A** as template which was obtained through BLAST results by taking **P75231** as query sequence and performing Protein BLAST against the protein sequence database.

Assessment of the Homology Model of Dihydroxy acetone kinase system protein: The validation of the final model was carried out using Ramachandran plot computed with PROCHECK, program by checking the detailed residue-by-residue stereo-chemical quality of a protein structure. The PROCHECK is used for stereo chemical assessment of the model. The criteria for analysis of stereochemistry of the model includes,

1) Main chain conformation in acceptable regions of the Ramachandran plot.
2) Planar peptide bonds.
3) Side chain conformations that correspond to those in rotamer library.
4) Hydrogen bonding of polar atoms if they are buried.
5) No bad atom-atom contacts.
6) No holes inside the structure.

Ramachandran Plot analysis by RAPPER

A Ramachandran plot (also known as a Ramachandran map or a Ramachandran diagram or a [Φ,Ψ] plot), developed by Gopalasamudram Narayana Ramachandran and Viswanathan Sasisekharan is a way to visualize dihedral angles Ψ and Φ of amino acid residues in protein structure. It shows the possible conformations of Φ and Ψ angles for a polypeptide. Hence, Ramachandran plot is a useful way of assessing the stereo chemical quality of a protein structure. The final refined model was further assessed by RAPPER server (ramachandran plot analysis), and the results show that Dihydroxy acetone kinase protein had 295 (96.3%)

ISSN: 0975-8585

residues in favoured region against (~98.0% expected), 35 (5.2%) residues in allowed region against (~2.0% expected) and 12 (3.5%) residues in outlier region as shown in **Figure-7** which shows that the final model is reliable.

Template structure (1BLS-A):

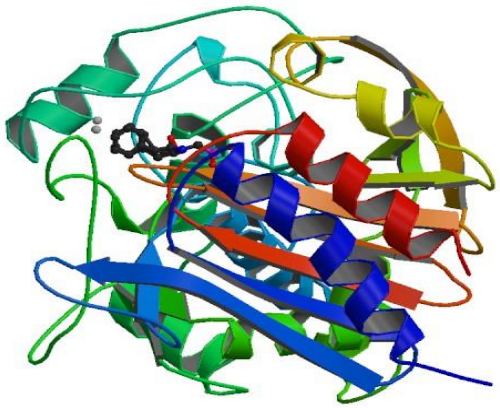


Figure-5:3d Structure Prediction of Dihydroxy acetone kinase system protein C by MOLSOFT:

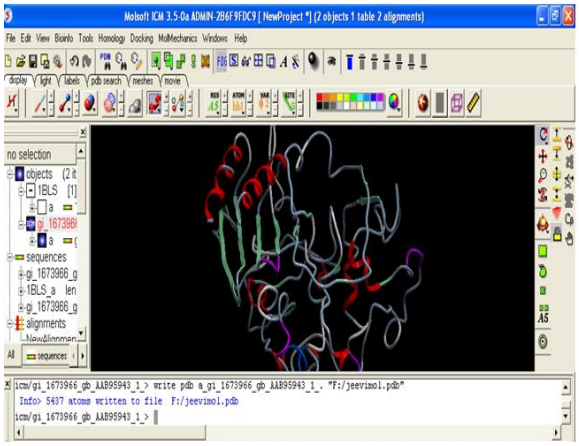
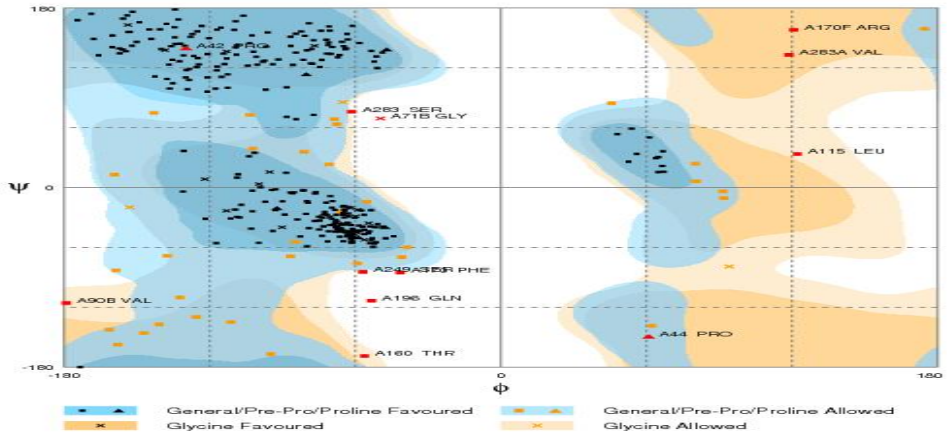


Figure-6: Shows Query (Dihydroxy acetone kinase) structure was modeled by using ICM molsoft by taking template as 1BLS, which is showing high similarity with query.

Ramachandran plot analysis



ISSN: 0975-8585

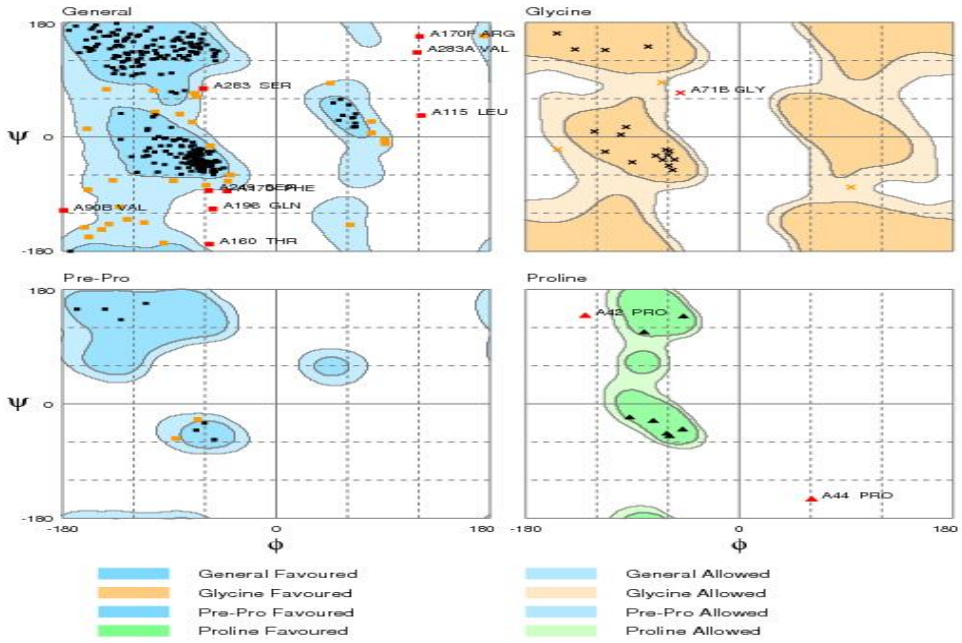


Figure-7: Protein validation study by RAPPER Server

Number of residues in favoured region (~98.0% expected): 295 (96.3%)
Number of residues in allowed region (~2.0% expected) : 35 (5.2%)
Number of residues in outlier region : 12 (3.5%)

CONCLUSIONS

The large scale genome sequencing projects have increased the availability of completely sequenced genomic and proteomic data in public domain. Use of the DEG database is more efficient than conventional methods for identification of essential genes and proteins and facilitates the exploratory identification of the most relevant drug targets in the pathogen.

The present study has thus led to the identification of several proteins that can be targeted for effective drug design and vaccine development against Mycoplasma pneumonia drugs developed against these will be specific to the pathogen, and therefore less or not toxic to the host. In the present study the numbers of essential gene products (Proteins) 26 are identified in Mycoplasma pneumoniae, by subtractive genomic approach. However these can be further increased, characterized and their role in the survival of the bacteria can be verified. Homology modeling of these targets will help identify the best possible sites that can be targeted for drug design by simulation modeling. Virtual screening against these novel targets might be useful in drug discovery.

In our study we have taken drug target as membrane protein Dihydroxy acetone kinase .The structure of Dihydroxy acetone kinase protein was modeled by taking template as 1BLS in MOLSOFT and model verification done by RAPPER.

REFERENCES

[1] Galperin MY, Koonin EV Searching for drug targets in microbial genomes. Curr Opin Biotechnol 10,1999, 571-578.
[2] Sakharkar KR, Sakharkar MK, Chow VT A novel genomics approach for the identifi cation of drug targets in pathogens, with special reference to Pseudomonas aeruginosa. In Silico Biology, 4,2004,355-360.
[3] Dutta A, Singh SK, Ghosh P, Mukherjee R, Mitter S, et al. In silico identifi cation of potential therapeutic targets in the human pathogen Helicobacter pylori. In Silico Biol 6, 2006,43-47.

[4] Overbeek R, Fonstein M, D'Souza M, Pusch GD, Maltsev N The use of gene clusters to infer functional coupling. Proc Natl Acad Sci USA, 96, 1999, 2896- 2901.
[5] Koonin EV How many genes can make a cell: the minimal-gene-set concept. Annu Rev Genomics Human Genetics 1, 2000, 99-116.
[6] Koonin EV Comparative genomics, minimal gene-sets and the last universal common ancestor. Nat Rev Microbiology ,1, 2003, 127-136.
[7] Gil R, Silva FJ, Peretó J, Moya A , Determination of the core of a minimal bacterial gene set. Microbiol Mol Biol Rev 68,2004, 518-537.
[8] Hutchison CA, Peterson SN, Gill SR, Cline RT, White O, et al.,Global transposon mutagenesis and a minimal Mycoplasma genome. Science 286,1999, 2165-2169.
[9] Himmelreich R, Hilbert H, Plagens H, Pirkl E, Li BC, et al., Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumonia*, Nucleic Acids Res 24,1996, 4420-4449.
[10] Wenzel R, Herrmann R , Cloning of the complete Mycoplasma pneumoniae genome. Nucleic Acids Res 17,1993, 7029-43.
[11] K.Praveen Kumar, U.Janardhan Reddy, A.Ranganadha Reddy, N.ShyamalaDevi ,Homology modeling and Docking Studies of MPT 51 Protein in Mycobacterial leprae International Journal of Chemtech Research,6(7),2014, 3864-3882 .
[12] Venkateswarulu TC, Kodali VP, Ranganadha Reddy A,John DB,Rahul RN,Indira M,Narayana AV,Comparative Studies of H5N1 Gene Segments with other Subtypes of Influenza- A Virus, Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5, 2014, 1417–1429.
[13] Ranganadha Reddy A, Venkateswarulu TC, JohnBabu D, M.Indira, Homology modeling studies of human genome receptor using modeller, swiss-model server and esypred-3d tools, International Journal of Pharmaceutical Sciences Review Research vol 30(5), 2015,1-6.
[14] .Séverine Altairac, "Naissance d'une banque de données: Interview du prof. Amos Bairoch". Protéines à la Une, August 2006. ISSN 1660-9824.
[15] Berman, H. Acta Crystallogr A: Foundations of Crystallography 2008, Vol 64,:pp88–95.
[16] Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.;Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.;Tasumi, M. Journal of Molecular Biology 1977, 112,:pp535–542.
[17] Berman, H. M.; Henrick, K.; Nakamura, H. Nat Struct Biol 2003, 10, 980.
[18] Ranganadha Reddy A, Sreedhara.R.Voleti, Lakshmi Padma Ch,Towards the predictionof a three-dimenional structure of human glp-1receptor. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4, 2013, 151-168.
[19] Ranganadha Reddy A, Venkateswarulu TC, JohnBabu D, M.Indira, Homology modeling studies of human genome receptor using modeller, swiss-model server and esypred-3d tools, International Journal of Pharmaceutical Sciences Review Research vol 30(5), 2015,1-6.
[20] Schapira M., Raaka B.M., Samuels H.H. and Abagyan R. In silico discovery of novel Retinoic Acid Receptor agonist structures BMC Structural Biology,2001, 1:1.
[21] Ranganadha Reddy A Salamuddin SK, Venkata Rami Reddy Ch, Homology modeling studies of ras protein in cancer Research Journal Pharmaceutical, Biological and Chemical Sciences , 5,(5) ,2014,273-283
[22] Ranganadha Reddy A,Venkateswarulu TC,Indira M, Narayana AV, Naga Lohita T, Sriharsha M,Identification of Membrane Drug Targets by Subtractive Genomic Approach in Mycoplasma Pneumonia, Research Journal of Pharmacy and Technology, 8(9), 2015, 1209-1216.