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RESEARCH ARTICLE

PHYLOGENETIC ANALYSIS AND HOMOLOGY MODELLING OF PROMISING LIGANDS FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Nucleic acids control heredity on a molecular level and enzymes like L-asparaginase catalyses the conversion of L-asparagine to L-aspartate. L Asparaginase catalyzes the hydrolysis of the non-essential amino acid L-Asn to L-Asp and ammonia and is widely used for the treatment of hematopoietic diseases such as acute lymphoblastic leukemia (ALL) and lymphomas. In the present work, we focus on the characteristic of the enzyme (family of glycosidase) L-Asparaginase that bears E.C.No 3.5.1.1 and its properties. Acute lymphoblastic leukemia is a cancer that starts from early version of white blood cells called lymphoblastic leukemia in the bone marrow and in silico tools were employed in the present study for the phylogenic analysis of the enzyme L-Asparaginase like CLUSTAL X, CLUSTAL W, GENE BEE, EMBOSS NEEDLE, MEGA and insilico docking studies of ligand structures obtained from Pubchem. Database 3.3 version, a new version of Easy Modeller (Easy Modeller 4.0) has been used for the modeling analysis. The target sequence utilized is a hypothetical human L-Asparaginase sequence bearing accession no: AAM28434.1. Four species were examined rat, chimpanzee, fruit fly & human with fish with percentages obtained as Humans with mouse = 92-94 % identity, Humans with chimpanzee =98 % identity, Humans with fruit fly = 42% identity &Humans with fish = 63 % identity. Ramachandran plots were analyzed via procheck and model no.3 was the best model plot.The data generated for the Insilico analysis of ALL (acute lymphoblastic leukemia) can also find further scope in other dreadful diseases.

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INTRODUCTION

Enzymes are the sparks that start the essential chemical reactions our bodies need to live. They are necessary for digesting food, for stimulating the brain, for providing cellular energy, and for repairing all tissues, organs, and cells. Humbart Santillo, in his book, Food Enzymes, quotes a Scottish medical journal that says it well: "Each of us, as with all living organisms, could be regarded as an orderly, integrated succession of enzyme reactions." Development of medical applications for enzymes has been at least as extensive as those for industrial applications, reflecting the magnitude of the potential rewards: for example, pancreatic enzymes have been in use since the nineteenth century for the treatment of digestive disorders. A major potential therapeutic application of enzymes is in the treatment of cancer. Asparaginase has proved to be particularly promising for the treatment of acute lymphocytic leukemia.

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Its action depends upon the fact that tumour cells are deficient in aspartate-ammonia ligase activity, which restricts their ability to synthesise the normally non-essential amino acid L-Asparagine (Clement, 1922). L-Asparaginase catalyzes the conversion of L-asparagine to L-aspartate (Selwood and Jaffe, 2011). Two related families of asparaginase (L-asparagine amide hydrolase, EC: 3.5.1.1) are designated type I and type II according to the terminology in Escherichia coli, which has both: L-asparaginase I is a low affinity enzyme found in the cytoplasm, while L-asparaginase II is a high-affinity periplasmic enzyme synthesized with a cleavable signal sequence.

This family describes L-Asparaginases (Müller, 1998) related to type II of E. coli. Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grows out of control.

Carcinomas are characterized by cells that cover internal and external parts of the body such as lung, breast, and colon cancer. In this study we focus on Acute L-Acute lymphocytic leukemia (ALL). This is also called acute lymphoblastic leukemia, is a cancer that starts from the early version of white blood cells called lymphocytes in the bone marrow (the soft inner part of the bones, where new blood cells are made). Leukemia cells usually invade the blood fairly quickly.

They can then spread to other parts of the body, including the lymph nodes, liver, spleen, central nervous system (brain and spinal cord), and testicles (in males). Other types of cancer also can start in these organs and then spread to the bone marrow, but these cancers are not leukemia. Cancer in children and adolescents is rare, although the overall incidence of childhood cancer, including ALL, has been slowly increasing since 1975.

Asparaginases are a cornerstone of treatment protocols for acute lymphoblastic leukemia (Broome, 1981; Appel *et al*., 2007) (ALL) and are used for remission induction and intensification treatment in all pediatric regimens and in the majority of adult protocols. Extensive clinical data have shown that intensive asparaginase treatment improves clinical outcomes in childhood ALL.

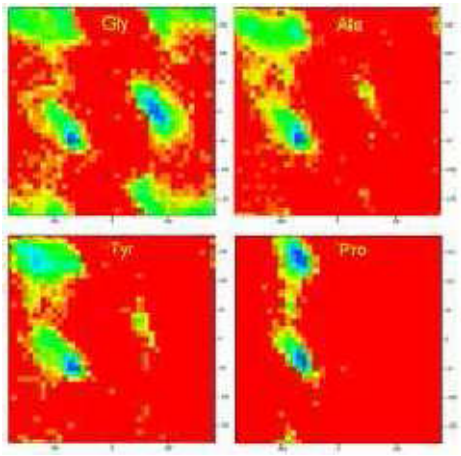

Fig. 1. Most stabilized Ramachandran plot

Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines computer science, statistics, mathematics, and engineering to study and process biological data (Kumar and Dudley, 2007). Bioinformatics is both an umbrella term for the body of biological studies that use computer programming as part of their methodology, as well as a reference to specific analysis "pipelines" that are repeatedly used, particularly in the fields of genetics and genomics. Common uses of bioinformatics include the identification of candidate genes and nucleotides (SNPs).

MATERIALS AND METHODS

For the present study, we used NCBI and BLAST PROTEIN servers and the program is able to work with ready sequences, imported from files of the following formats:

EMBL/SWISSPROT, NBRF/PIR, Pearson (Fasta), GCG/MSF (Pileup), Clustal, GCG9/RSF and GDE. MEGA, Molecular Evolutionary **G**enetics **A**nalysis was used for statistical analysis of molecular evolution and for constructing phylogenetic trees (Swofford *et al*., 1996).

A new version of EasyModeller (EasyModeller 4.0) has been recently released and available for free download for Linux and Windows OS. Secondary structure of proteins can be predicted using web based tools such as

- J PRED
- NN PREDICT
- 3DPSSM
- CAST-P
- GENO 3D and software's such
- RASMOL or PHYMOL

General commands and format for loading a molecule

load [format] <filename> Load a molecule

pdb -Protein Databank
zap -Delete molecule
exit -Exit from RasMol
help -[topic [subtopic]] Display on-line help topic

select- <expression> Update part of molecule
restrict- <expression> Display only part of mol.

All atoms
cys - Atoms in cysteine
hoh - Atoms in water molecules
as - Atoms in asparagine or aspartic acid

120 - Atoms at residue 120 of all chains
p - Atoms in chain P
n - Nitrogen atoms

cys.sg -Sulphur atoms in cysteine residues
ser70.c? -Carbon atoms in serine-70
hem*p.fe -Iron atoms in the Heme of chain P
A -Atoms in alternate conformation A
4 -All atoms in model 4

The multiple sequence alignment is done using Clustal X-software and the main steps are:

- In order to make a multiple sequence alignment using Clustal X, the 10 sequences should be in FASTA format. Save them in a word or notepad form on the system.
- Pull down the File-menu, and choose Load Sequences menu item. Navigate to the folder (subdirectory) that contains the input file (text-file containing the sequences in FASTA format), and choose that file. Sequences should appear in the ClustalX window.
- Before aligning the sequences, it should be ensured that the output format options (from Alignment -> output format options) are set correctly. To continue phylogenetics analysis using Phylip package select PHYLIP format.
- Note: Always save the Clustal formatted sequence alignment.
- In order to make the actual alignment, select "Do complete alignment" from the menu Alignment. At that point Clustal

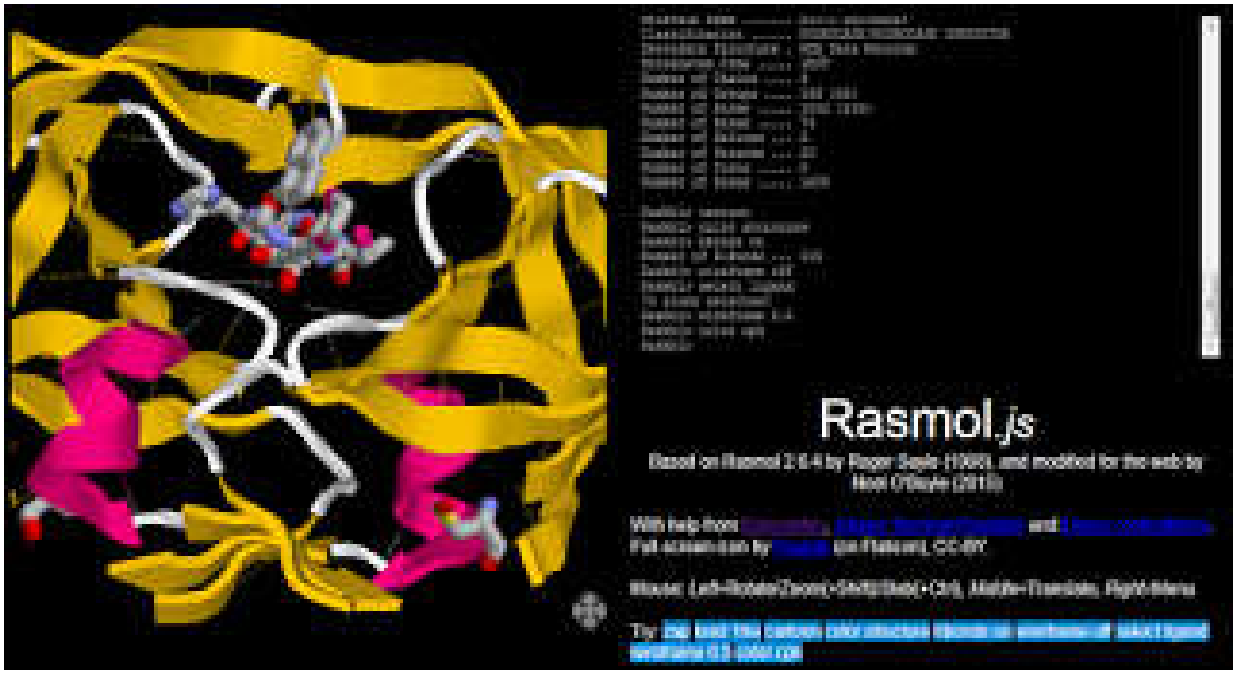

Fig. 2. Rasmol showing structure of target protein generated

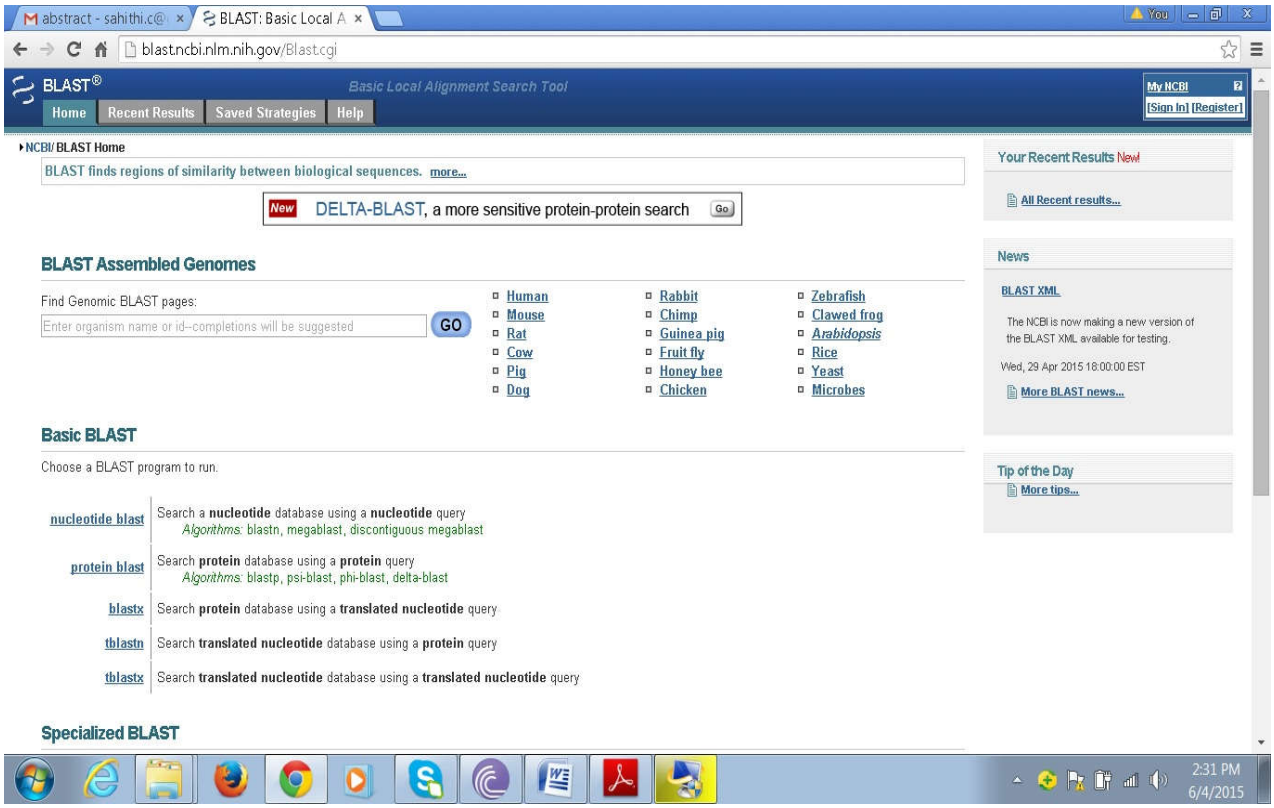

Fig. 3. A comparison of the e-values of all the organisms

X asks for output file name hasbeen successfully calculated various parameters can be analyzed. The sequence alignment is automatically saved in those files once the alignment is ready.

- After the alignment extract human L Asparaginase sequence in FASTA format from NCBI.
- Paste the sequence into 3d pssm window.
- Submit the query.
- Analyze the results according to requirement. The generated structures can be visualized using Rasmol.

RESULTS AND DISCUSSION

When gene 3d was performed following results were obtained: various Ramachandran Plots were observed as well as the best model for our target protein was selected based on the stability

results of the models. The stereo chemical properties of the modeled protein or query protein were analyzed via Procheck analysis in the plots obtained plot no 3 consisting of 80% accuracy in the most favorable region and very few residues in the dissolved region was considered the best model plot and its corresponding model no3 was the most optimum score. When 3d pssm was performed following results were obtained as depicted below. These results consisted of various templates generated for our target sequence.

Table 1. Mouse Buttons for PC

Key	Function
Left Button	Rotate X-Y
Right Button	Translate X-Y
Shift Left Button	Zoom
Shift Right Button	Rotate Z
Control Left Button	Z-Clipping (Slab)

The target sequence utilized is a hypothetical human L Asparaginase sequence bearing accession no: AAM28434.1. When homology modeling was performed initially with J pred following results were obtained. Many hits were provided for our query sequences which which are called matches or the target sequence utilized is a hypothetical human L Asparaginase sequence bearing accession no: AAM28434.1

DISCUSSION

Four different species were considered for the Phylogenetics analysis and the respective sequences in their BLAST formats were extracted from the main server NCBI.

They were

- Human (Homo sapiens)
- Chimpanzee (Pan Troglodytes)
- Fish (Daniorerio)
- Fruit fly (Drosophila melanogaster)

These sequences when run in blast yielded several catalytic nucleophillic sites, conserved Domains and conserved regions.

NOTE: "MINIMUM IS THE E-VALUE MORE SIGNIFICANT IS THE DATA**".** This means that the organisms having lower e-value are grouped together since they form the natural or closer neighbors.

Conclusion

The present study was carried out to examine the various aspects of phylogenetics and simulation models. Homology modelling played a crucial role in the study to understand data acquisition, analysis, algorithm development and dissemination through computational methodologies. All the various software tools utilized in this work have a user friendly environment and access to different underlying principles of phylogeny and molecular modelling strategies. Phylogenetics allowed the assessment of the evolutionary relationships of various organisms exploiting the fact that the basic enzyme present in all of them i.e. L- Asparaginase is the same. Four species were examined viz., rat, chimpanzee, fruit fly & human with fish.

The percentages obtained from the phylogenetic assessment are: Humans with rat = 92-94 % identity, Humans with chimpanzee =98 % identity, Humans with fruit fly = 42% identity and Humans with fish = 63 % identity. The L-Asparaginase analysis showed fluctuating patterns of consistency in the organisms utilized for study. Homology modeling and the various simulations allowed easy manipulation of the structural and stereo chemical properties of the hypothetical human protein consisting of L -Asparaginase leading to successful generation of three dimensional structure for our query. The software tools used for analysis are Clustal X, Clustal W, Gene bee & Modeller 9.14 v and 3d structure were obtained from Rasmol, Cast P& J pred. Together Phylogenetics and molecular modeling strategies would help in the design and fabrication of novel proteins with beneficial characteristics which would help discard the ambiguity and abnormalities of harmful proteins aroused due to various discrepancies during formation of proteins. A number of cases can register its use such as those of viral proteins in HIV and various cancerous conditions. The data generated for the insilico analysis of ALL (acute lymphoblastic leukemia) cab also find further scope in other dreadful diseases.

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