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A comparative protein function analysis database of different *Leishmania* strains

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Abstract:
A complete understanding of different protein functional families and template information opens new avenues for novel drug development. Protein identification and analysis software performs a central role in the investigation of proteins and leads to the development of refined database for description of proteins of different *Leishmania* strains. There are certain databases for different strains that lack template information and functional family annotation. Rajendra Memorial Research Institute of Medical Sciences (RMRIMS) has developed a web-based unique database to provide information about functional families of different proteins and its template information in different *Leishmania* species. Based on the template information users can model the tertiary structure of protein. The database facilitates significant relationship between template information and possible protein functional families assigned to different proteins by SVMProt. This database is designed to provide comprehensive descriptions of certain important proteins found in four different species of *Leishmania* i.e. *L. donovani, L. infantum, L. major* and *L. braziliensis.* A specific characterization information table provides information related to species and specific functional families. This database aims to be a resource for scientists working on *proteomics.* The database is freely available at http://biomedinformri.org/calp/.

Keywords: *Leishmania*, database, protein function, functional family, CPL, Computational proteomics of *Leishmania*

Background:
Kala-azar or Leishmaniasis is identified by clinical syndromes caused by obligate intracellular protozoa of the genus *Leishmania* and transmitted from one host to another by the bite of blood sucking sand fly vectors **[1]**. The genomes of three species have been sequenced. There are relatively few species-specific differences in gene content between the sequenced genomes, but nearly 8% of the genes appear to be evolving at different rates **[2]**. Knowledge about protein function is essential in the understanding of biological processes **[3]**. No computational functional analysis of different proteins of *Leishmania* is available till date. As the gap between the amount of sequence information and functional characterization widens, increasing efforts are being intended for the construction of databases. For scientist, it is therefore helpful to have a single data collection point, which integrates research interrelated data from diverse domains. Large scale of protein sequences is available at the National Center for Biotechnology Information (NCBI) protein database **[4]** and supplementary data in the published literature. In silico analysis gives us an idea on the role of different proteins in replication, survival and spread in the host **[5]**. Computational proteomics of *Leishmania* (CPL) involves the general tasks related to analysis of any novel sequences, such as functional annotation and template information of the sequences. Support vector machine (SVM) is a useful classifier for predicting the functional classes of distantly related proteins **[6, 7]**. The function of a protein depends on its tertiary structure. The structure and function of a protein gives much more insight of the protein than its sequence **[8]**. Structural genomics are yielding many protein structures that have unknown function. Nevertheless, successive experimental investigation is costly and time-consuming, which makes computational methods for predicting protein function very attractive **[9]**. Therefore, a number of methods for the computational prediction of protein structure from its sequence have been proposed. The simulated model of the protein structure refers to the construction of an atomic-resolution model of the target protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein (i.e. template) **[10]**. The critical first step in homology modeling is the discovery of the best template structure based on which a tertiary structure will be modeled **[11]**. Considering the biological significance of protein and with the aim of providing easy access to large and growing volume of data, we have developed a repository for most common proteins in which user can get the information about the template and functional family of protein. As drug resistance problem persists in case of Leishmaniasis, template information will help further modeling and analysis of different essential proteins which would lead to the discovery of novel lead compounds.

Methodology:
The large scale of protein sequences have been reported in the NCBI protein database and supplementary data in the published literature. The commonly available virulent sequences of *Leishmania* have been downloaded from the National Center for Biotechnology Information (NCBI) and GeneDB **[12]**. Different strains of the same species from samples collected from diverse location or at different times may have completely identical sequences. Redundancy and repetition in protein sequences has been carefully removed by using ALIGN software to obtain a unique dataset **[13]**. Exactly matching sequences taken from multiple sources were eliminated while constructing the dataset. The raw dataset was preprocessed to remove the sequence smaller than 50bp while analyzing with different software.

Database design:

A rational database was constructed in MySQL for storage and query of data. It includes two key entities namely molecular function and template structure which fetches the probable function and most appropriate virtual structure of the protein. The database consists of three layers: the basal layers', 'Application layer' and 'UI' layer. These layers is developed using Php, CSS and JavaScript. The information's are managed in protein level to provide timely and general view of the data. The data and information have been stored in MySQL relational database. Meta information for different types of biological data is placed as individual table in this layer **(Figure 1)**.

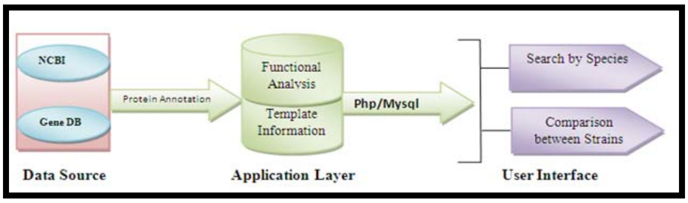
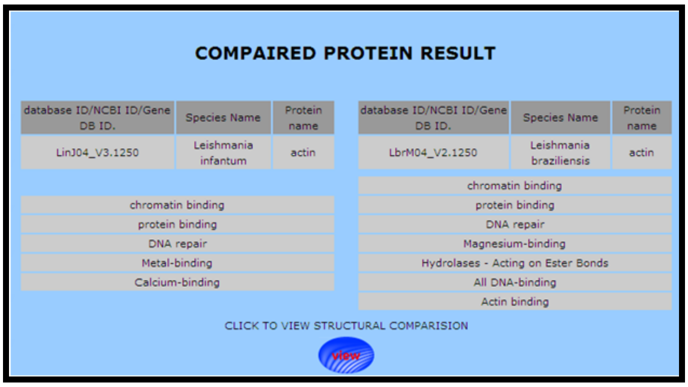


Figure 1: System architecture

Database features:

The data/information store in database can be accessed in very simple way like Search by species name and protein functional family: The user can enter the desired species name to access the common protein present in other strain of that species e.g. if somebody enter the sp. name *L.donovani* then it will show all protein of *L.donovani* which is also present in other three strain i.e. *L.major*, *L.infantum* and *L. braziliensis*).The user can select the different protein functional family to find out the proteins that possesses the same function **(Figure 2)**. The user can compare the proteins of different species and their functional families. The user can also compare tertiary structure of the templates **(Figure 3)**.



COMPAIRED PROTEIN RESULT

database ID/NCBI ID/Gene DB ID.	Species Name	Protein name
LinJ04_V3.1250	Leishmania infantum	actin

database ID/NCBI ID/Gene DB ID.	Species Name	Protein name
LbrM04_V2.1250	Leishmania braziliensis	actin

chromatin binding
protein binding
DNA repair
Metal-binding
Calcium-binding

chromatin binding
protein binding
DNA repair
Magnesium-binding
Hydrolases - Acting on Ester Bonds
All DNA-binding
Actin binding

CLICK TO VIEW STRUCTURAL COMPARISION

Figure 2: Typical screenshots of the Database showing the functional family of two compared protein with Database id, species name and protein name.

Results and Discussion:

Identification of diverse protein functions may facilitates a mechanistic understanding of different proteins and opens novel means for drug development. Nearly 25 important proteins of each species have taken into consideration. Our study from SVMProt suggests that the proteins of different strains are having lyases - carbon-oxygen lyases, actin binding, DNA-binding, hydrolases - acting on ester bonds, magnesium-binding, calcium-binding, copper-binding, metal-binding, DNA repair, zinc-binding, transmembrane and all lipid-binding group of functional family. But most of the proteins commonly belong to all lipid-binding proteins, zinc-binding and metal-binding functional families. It is analyzed that the most of the homologous amino acid sequences belong to same functional group. But change in amino acid composition may affect the functional properties of the proteins. For example the analysis of RAD51 protein suggests that mutation of RAD51 protein of *L. braziliensis* may change the availability of some functional groups **(Table 1 see Supplementary material)**. From multiple sequences alignment of RAD51 protein of *L. braziliensis*, it is analyzed that the mutation of glycine to threonine , arginine to glutamine, serine to valine, valine to methionine, alanine to cysteine, glutamic acid to valine, proline to phenylalanine, glutamine to proline, serine to glycine , aspartic acid to glycine, methionine to valine, cysteine to tyrosine and serine to alanine at different position may lack the availability of aptamer-binding protein, outer membrane and RNA-binding functional family and availability of lyases - carbon-oxygen lyases, actin binding, all lipid-binding proteins group of functional family. Mutation of arginine to glutamine at 41st position and valine to isoleucine at 321st position may increase the availability of oxidoreductases -acting on the CH-CH group of donors and manganese-binding functional family **(Figure 4)**. In *L. donovani* the insertion of proline at 43rd position and mutation of glutamic acid to aspartic acid may increase the frequency of DNA recombination and mRNA splicing functions.

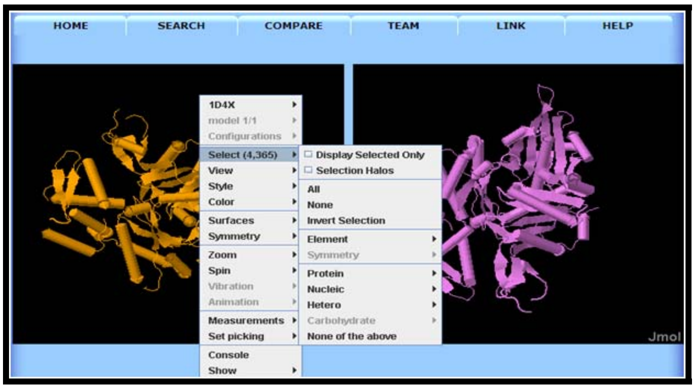


Figure 3: Structural (template) comparison of the two proteins

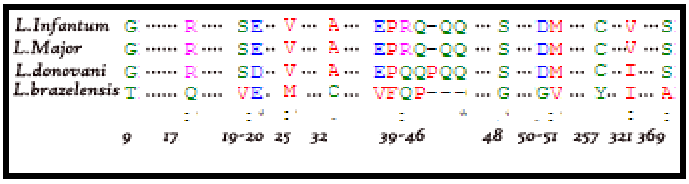

L.Infantum G R SE V A EPRQ-QQ S DM C V S
L.Major G R SE V A EPRQ-QQ S DM C V S
L.donovani G R SD V A EPQQPQQ S DM C I S
L.brazelensis T Q VE M C VFQP--- G GV Y I A
9 17 19-20 25 32 39-46 48 50-51 257 321 369

Figure 4: Representation of Multiple sequence alignment of RAD51 protein

Utility:

With the aim of providing easy access to large and growing volume of data, a database of most common protein is developed. This is the first web resource which provides the common protein sequence of four strains as well as their functional classes for comparison. The database has been analyzed, organized and integrated to develop a user friendly interface. The web interface enables the user to execute a quick and efficient search and comparison. The database will be an extremely useful resource for computational and experimental biologists working in *Leishmania* proteomics and related areas.

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[12] http://www.genedb.org/Homepage
[13] http://www.ebi.ac.uk/Tools/emboss/align/index.html

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Supplementary material:

Table 1: Availability of Functional family **in** RAD51 protein

Functional family	*L. braziliensis*	*L.infantum*	*L.donovani*	*L.major*
Zinc-binding	✓	✓	✓	✓
DNA repair	✓	✓	✓	✓
RNA-binding Proteins	✗	✓	✓	✓
Metal-binding	✓	✓	✓	✓
Lyases - Carbon-Carbon Lyases	✓	✓	✓	✓
All DNA-binding	✓	✓	✓	✓
Outer membrane	✗	✓	✓	✓
Aptamer-binding protein	✗	✓	✓	✓
All lipid-binding proteins	✓	✗	✗	✗
Actin binding	✓	✗	✗	✗
Lyases - Carbon-Oxygen Lyases, Actin binding	✓	✗	✗	✗
Oxidoreductases - Acting on the CH-CH group of donors	✓	✗	✓	✗
Manganese-binding	✓	✗	✓	✗
DNA recombination	✗	✗	✓	✗
mRNA slicing	✗	✗	✓	✗