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COMPARATIVE GENOMIC STUDIES AND IN-SILCO STRATEGIES ON *LEISHMANIA BRAZILENSIS, LEISHMANIA INFANTUM* AND *LEISHMANIA MAJOR*: CONSERVED FEATURES, PUTATIVE FUNCTIONS AND POTENTIAL DRUG TARGET

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Abstract
Leishmaniasis is a parasitic disease found largely in the tropics, which the World Health Organization has estimated infects 12 million people worldwide each year. More recently cases have been reported in Europe among intravenous drug users with HIV. At least 20 *Leishmania* species infect humans. New world parasite *Leishmania. braziliensis* is the causative agent of mucocutaneous Leishmaniasis. The old world species *Leishmania. major* and *Leishmania. infantum*, which are present in Africa, Europe and Asia, are parasites that cause cutaneous and visceral Leishmaniasis respectively. Aim of this Study is determination of major common genes and Protein identified Gene location on each of the chromosomes, and identification of a common protein drug target Promastigote surface antigen with available lead molecule acetylglucosamine (6-(acetylamino)-6-deoxyhexopyranose) and docking studies on those considered *Leishmania* species.

Keywords: Leishmaniasis, Promastigote surface antigen, Acetylglucosamine

Introduction

Leishmaniasis is a parasitic disease found largely in the tropics, which the World Health Organization has estimated infects 12 million people worldwide each year. More recently cases have been reported in Europe among intravenous drug users with HIV. At least 20 *Leishmania* species infect humans. New world parasite *Leishmania. braziliensis* is the causative agent of mucocutaneous Leishmaniasis. The old world species *Leishmania. major* and *Leishmania. infantum*, which are present in Africa, Europe and Asia, are parasites that cause cutaneous and visceral Leishmaniasis respectively (Christopher S Peacock *et al,* 2007). Three type of Leishmaniasis Visceral Leishmaniasis: The most serious form in which parasites leave the inoculation site and proliferate in liver, spleen and bone marrow, resulting in host immuno-suppression and ultimately death in the absence of treatment. Cutaneous Leishmaniasis: In which parasites remain at the site of infection and cause localized long-term ulceration. Mucocutaneous Leishmaniasis: A chronic destruction of mucosal tissue that develops from the cutaneous disease in less than 5% of affected individuals.

In India Visceral Leishmaniasis (VL), also known as kala-azar, black fever, and Dumdum fever is the most severe form of Leishmaniasis. (Lira R, Sundar S, Makharia A, Kenney R, Gam A, *et al,* 1999)Leishmaniasis is a disease caused by protozoan parasites of the *Leishmania* genus. This disease is the second-largest parasitic killer in the world (after malaria), responsible for an estimated 500,000 infections each year worldwide (William D, Berger, Timothy G *et al,* 2006). Diagnosis for each of these types of Leishmaniasis involves taking a scraping from a lesion, preparing it in a laboratory, and examining it under a microscope to demonstrate the causative protozoan with a skin test (similar to that test for TB). Phlebotomine sand flies are the only known natural vectors of *Leishmania* more than 400 Phlebotomine species are described fewer than fifty are known to be involved in the transmission cycle of these parasites some vectors species are highly

restricted to the species of *Leishmania* that they transmit in nature.

The objective of our study was determination of major common genes and protein identified gene location on each of the chromosomes, and identification of a common protein drug target Promastigote surface antigen with available lead molecule acetylglucosamine (6-(acetylamino)-6-deoxyhexopyranose) and docking studies on those considered *Leishmania* species. (Marsden, P.D. Mucosal Leishmaniasis, *et al*, 1986)

Material and Methods

Species for the genomic studies and their in-silco strategies

Leishmania. major Friedlin

Leishmania.major, the reference strains (MHOM/IL/80/Friedlin, zymodeme MON-103) it was first sequenced as part of a multi-centre collaboration. The genome has been manually annotated and so far more than 8,000 genes have been identified in the ~33.6Mb haploid genome which is spread over 36 chromosomes the database is undergoing continual manual annotation and citation.

Leishmania infantum

The genome of *Leishmania. infantum* is the second *Leishmania* species to be sequenced at the Sanger Institute. The clone used was generated a whole genome shotgun sequence of *Leishmania. infantum* JPCM5 (MCAN/ES/98/LLM-877). This pathogen is the causative agent of visceral Leishmaniasis in the Mediterranean basin. Gene prediction and annotation has been partly generated and analyzed by comparison to the *Leishmania. major* genome as sequencing and annotation are in progress, the data is continually updated.

Leishmania. braziliensis

Leishmania. braziliensis the third *Leishmania* species to be sequenced at the Welcome Trust Sanger Institute. Responsible for causing localized cutaneous lesions in affected people. *Leishmania. braziliensis* can also result in a more destructive, progressive infection in mucosal tissue Karyotyping of *Leishmania. braziliensis* has shown that it has 35 chromosomes compared to the 36 present in old world species. The difference in chromosome number is due to the fusion of chromosomes 20 and 34 to make a single chromosome in *Leishmania. braziliensis*.

Results and Discussion

Summary of Genomes:

The genomic studies and their in-silco strategies of all the three species of *Leishmania* been shown in the Table 1

Common Genes and there locus:

One of the studies involved in the insilico-detection of common genes and their locus on all the chromosomes with the three *Leishmania* species is been tabulated from chromosome number 1 to chromosome number 36. An example or sample of common genes located on the chromosome 1 and 2 is shown in table 2 and table 3 respectively.

Common Protien target in all the three species of *Leishmania*:

PSA (Promastigote surface antigen) is one of the major classes of membrane proteins present at the surface of the parasitic protozoan *Leishmania.* (El- Sayed, N.M. *et al*, 2005). PSA is a *Leishmania* family of membrane-bound or secreted proteins. Its Basic Local Alignment Search was done against NCBI's Genbank Database and Multiple Sequence Alignment was carried out using ClustalW an online multiple alignment tool. The below figure 1 shows the result of the Protein BLAST in all three *Leishmania* and figure 2 shows the alignments of the Promastigote surface antigen sequence with three *Leishmania* species. (Croft SL, Coombs GH. *et al*, 2003)

Table 1: In-silco Genome strategies of all the three species of *Leishmania*

Genomic Attributes	*Leishmania major*	*Leishmania infantum*	*Leishmania. braziliensis*
Chromosome number	36	36	35
Size(bp)	32,816,678	32,134,935	32,005,207
Overall G+C content (%)	59.7	59.3	57.76
Coding genes	8,298	8,154	8,153
Pseudo genes	97	41	161

Table 2: Common Genes and there locus on Chromosome Number 1

Product Name	*Leishmania major*	*Leishmania infantum*	*Leishmania. braziliensis*
phosphoglycan beta 1,3 galactosyltransferase 3	LmjF02.0010	LinJ02.0140	LbrM02_V2.0010
cytochrome b-domain protein, putative	LmjF02.0050	LinJ02.0020	LbrM02_V2.0070
exportin T (tRNA exportin)-like protein	LmjF02.0110	LinJ02.0090	LbrM02_V2.0120
phosphatidylinositol 3-kinase-like protein	LmjF02.0120	LinJ02.0100	LbrM02_V2.0130
small GTP binding protein rab6-like protein	LmjF02.0260	LinJ02.0180	LbrM02_V2.0290
protein kinase, putative	LmjF02.0290	LinJ02.0210	LbrM02_V2.0340
ABC1 transporter, putative	LmjF02.0300	LinJ02.0220	LbrM02_V2.0350
casein kinase II, alpha chain, putative	LmjF02.0360	LinJ02.0280	LbrM02_V2.0390
proteasome regulatory non-ATPase subunit 6, putative	LmjF02.0370	LinJ02.0290	LbrM02_V2.0400
FtsJ-like methyltransferase, putative	LmjF02.0380	LinJ02.0300	LbrM02_V2.0410
ubiquitin-conjugating enzyme e2, putative	LmjF02.0390	LinJ02.0310	LbrM02_V2.0420
RNA-editing complex protein MP81, putative	LmjF02.0410	LinJ02.0330	LbrM02_V2.0430
protein kinase, putative	LmjF02.0570	LinJ02.0490	LbrM02_V2.0540
ARP2/3 complex subunit, putative	LmjF02.0600	LinJ02.0520	LbrM02_V2.0580
gamma-glutamyl phosphate reductase-like protein	LmjF02.0630	LinJ02.0550	LbrM02_V2.0610
mitochondrial carrier protein, putative	LmjF02.0670	LinJ02.0590	LbrM02_V2.0650
metallo-peptidase, Clan MA(E), Family M3	LmjF02.0740	LinJ02.0660	LbrM02_V2.0670

Table 3: Common Genes and there locus on chromosome number 2 as an example

Product Name	*Leishmania major*	*Leishmania infantum*	*Leishmania. braziliensis*
phosphoglycan beta 1,3 galactosyltransferase 3	LmjF02.0010	LinJ02.0140	LbrM02_V2.0010
cytochrome b-domain protein, putative	LmjF02.0050	LinJ02.0020	LbrM02_V2.0070
exportin T (tRNA exportin)-like protein	LmjF02.0110	LinJ02.0090	LbrM02_V2.0120
phosphatidylinositol 3-kinase-like protein	LmjF02.0120	LinJ02.0100	LbrM02_V2.0130
small GTP binding protein rab6-like protein	LmjF02.0260	LinJ02.0180	LbrM02_V2.0290
protein kinase, putative	LmjF02.0290	LinJ02.0210	LbrM02_V2.0340
ABC1 transporter, putative	LmjF02.0300	LinJ02.0220	LbrM02_V2.0350
casein kinase II, alpha chain, putative	LmjF02.0360	LinJ02.0280	LbrM02_V2.0390
proteasome regulatory non-ATPase subunit 6, putative	LmjF02.0370	LinJ02.0290	LbrM02_V2.0400
FtsJ-like methyltransferase, putative	LmjF02.0380	LinJ02.0300	LbrM02_V2.0410
ubiquitin-conjugating enzyme e2, putative	LmjF02.0390	LinJ02.0310	LbrM02_V2.0420
RNA-editing complex protein MP81, putative	LmjF02.0410	LinJ02.0330	LbrM02_V2.0430
protein kinase, putative	LmjF02.0570	LinJ02.0490	LbrM02_V2.0540
ARP2/3 complex subunit, putative	LmjF02.0600	LinJ02.0520	LbrM02_V2.0580
gamma-glutamyl phosphate reductase-like protein	LmjF02.0630	LinJ02.0550	LbrM02_V2.0610
mitochondrial carrier protein, putative	LmjF02.0670	LinJ02.0590	LbrM02_V2.0650
metallo-peptidase, Clan MA(E), Family M3	LmjF02.0740	LinJ02.0660	LbrM02_V2.0670

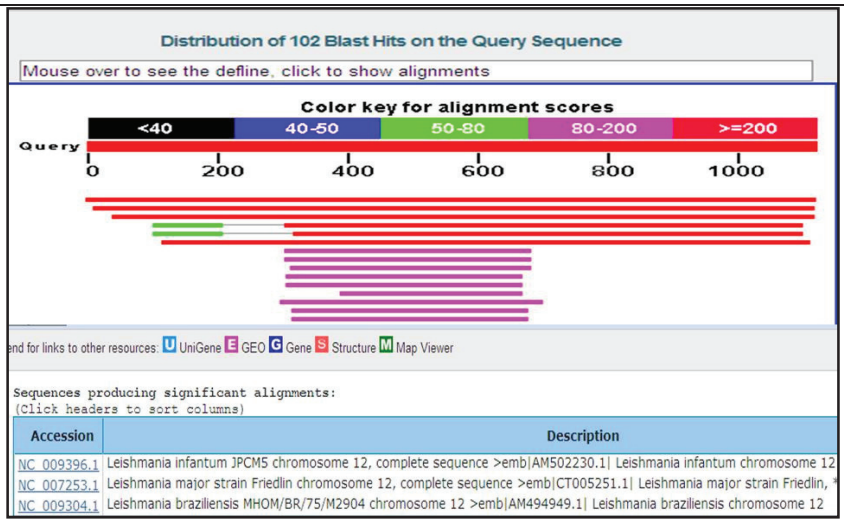

Fig. 1: The result of the Protein BLAST against NCBI's GenBank Database

```
inf             CDACQGSGEHIDPRNRCGRCSGNKTVEVDASVQVVVEKGMAHRQRITFPRMADEELGVER 170
maj             CPVCKGKGTNLREEDKCVSCRGQQIIKDKKVFEVMVEKGMHRGDSVTFSGEGDQIPGVKL 236
braz            CESQKVTGDVIG-VDLGTTYSCVATMDGDKARVLENSEGFRTTPSVVAFKGSEKLVGLAA 78
                *   : .*  :   :          :. .    :  .:*:     :.    .::  *:

inf             AGDFVVVLQQVKHDVFTREDCDLHMQRHLSLAEALCGFQFKFTHLDGRELVVRQARGTIT 230
maj             SGDIIIILDQKPHQTFIRKGDHLFLEQTISLAEALTGFSLNITQLDGRELAISSTAGTII 296
braz            KRQAITNPQSTFYAVKRLIGRRFEDEH---IQRDIKNVPYKIVRAGNGDAWVQDGNGKQY 135
                  : :   :.  : .    .  :  ::   : . : ..  ::.: .. :  : .  *.

inf             KPGDVKCVIGEGMPLHKQANR-FGNLIIEFNVKYPDRIEAGQLQLLREALPPPKSVDVAA 289
maj             DPANMYSVSREGMPVAHTGGMERGDLIIRFQVVFPKTLRQGCVPELRKMLGYPQ----QP 352
braz            SPSQVGAFVLEKM--KETAQNFLGHTVSNAVVTCPAYFNDAQR--------------QA 178
                .*.:: ..  * *   . .    *. : .  *  *  :. .                  .

inf             DNEAGDVCYVTREDLSVLEEEIKKDEEAEEENEG--PQTGCAAQ 331
maj             PAKDGAEQYTLQESHIDLEKEARRNAYDDDGDQPRVQTAGCAQQ 396
braz            TKDAGTIAGLNVIRVVNEPTAARCG------------------- 203
                  . *               : .
```

Fig. 2: Multiple sequence alignment of Promastigote surface antigen conserved regions with three *Leishmania* species.

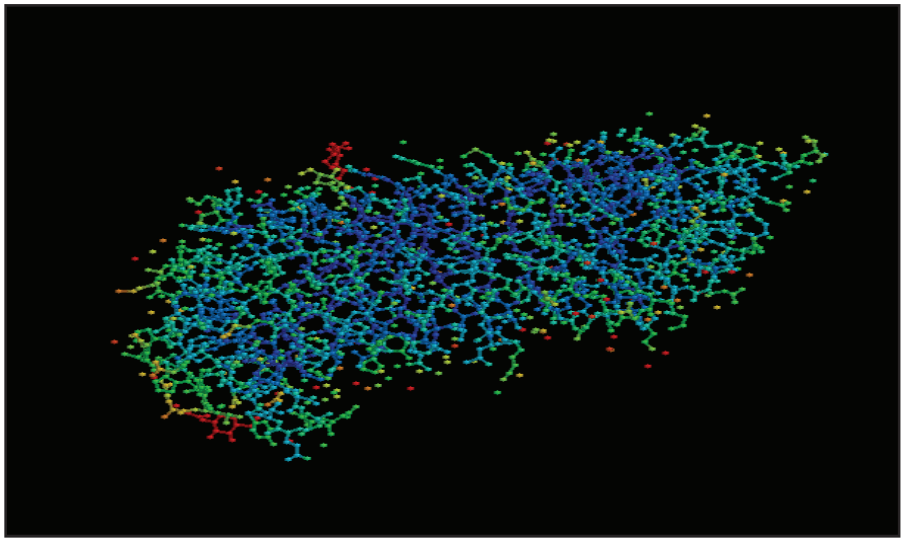

Fig. 3: The Ball and Stick 3-Dimensional view of Promastigote surface antigen (PSA) in Arguslab workspace window

Docking Results:

Clustering the final poses = 44 final unique configurations. Number of local searches that succeeded in locating new minims= 3. Re-clustering the final poses = 44 final unique configurations. Best Ligand Pose: energy = -5.56111 kcal/mol. Docking run: elapsed time=11 seconds.

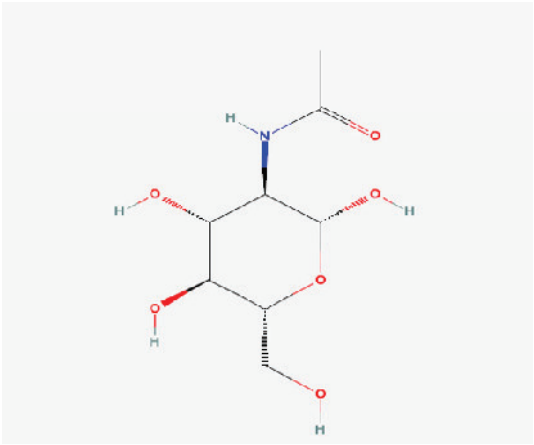

Fig. 4: Two-Dimensional structure of the Inhibitor Acetylglucosamine: (6-(acetylamino)-6-deoxyhexopyranose)

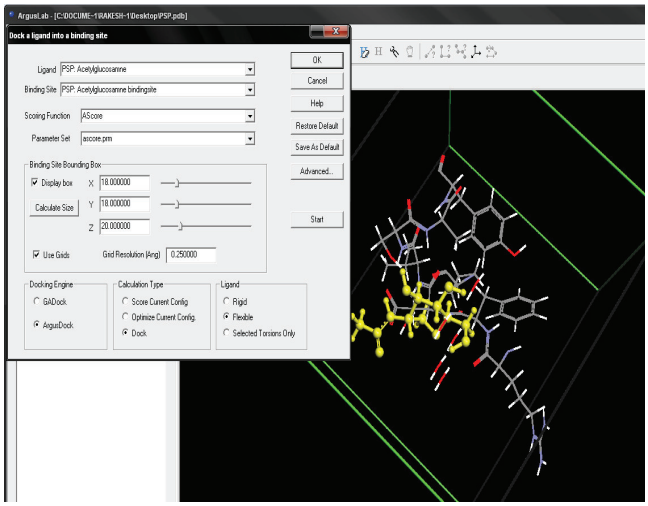

Fig. 5: Optimization of the Ligand and its Binding site in Arguslab with grid setting.

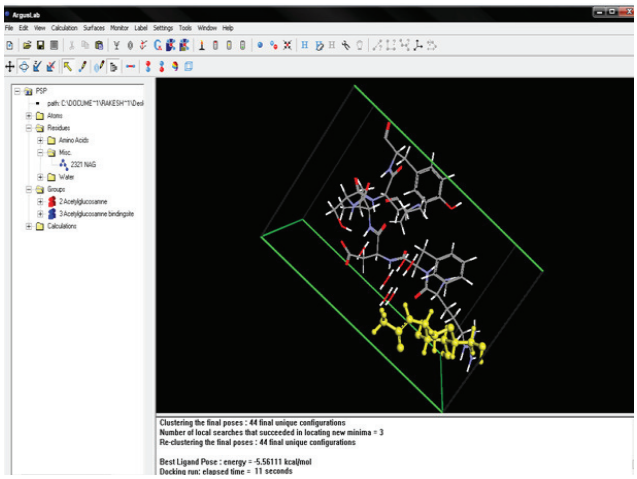

Fig. 6: Results of Docking with Ligand(Acetylglucosamine) and Receptor (Promastigote surface antigen) with elapsed time and best ligand pose energy.

Conclusion

Comparisons of the complete genomes of three species of *Leishmania* allowed revealing a novel genus-specific gene. A common drug target was identified within 3 species. Identification of a few genes that are either species-specific or under positive selective pressure provides a comprehensive and manageable resource to target efforts in identifying parasite factors that influence infection. Conversely, factors that are unique to the *Leishmania* genus but common to all species may be used as potential drug targets or vaccine candidates.

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