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DSFL database: A hub of target proteins of *Leishmania* sp. to combat leishmaniasis

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ABSTRACT

Leishmaniasis is a vector-borne chronic infectious tropical dermal disease caused by the protozoa parasite of the genus *Leishmania* that causes high mortality globally. Among three different clinical forms of leishmaniasis, visceral leishmaniasis (VL) or kala-azar is a systemic public health disease with high morbidity and mortality in developing countries, caused by *Leishmania donovani*, *Leishmania infantum* or *Leishmania chagasi*. Unfortunately, there is no vaccine available till date for the treatment of leishmaniasis. On the other hand, the therapeutics approved to treat this fatal disease is expensive, toxic, and associated with serious side effects. Furthermore, the emergence of drug-resistant *Leishmania* parasites in most endemic countries due to the incessant utilization of existing drugs is a major concern at present. Drug Search for Leishmaniasis (DSFL) is a unique database that involves 50 crystallized target proteins of varied *Leishmania* sp. in order to develop new drugs in future by interacting several anti-parasitic compounds or molecules with specific protein through computational tools. The structure of target protein from different *Leishmania* sp. is available in this database. In this review, we spotlighted not only the current global status of leishmaniasis in brief but also detailed information about target proteins of various *Leishmania* sp. available in DSFL. DSFL has created a new expectation for mankind in order to combat leishmaniasis by targeting parasitic proteins and commence a new era to get rid of drug resistance parasites. The database will substantiate to be a worthwhile project for further development of new, non-toxic, and cost-effective antileishmanial drugs as targeted therapies using *in vitro/in vivo* assays.

1. Introduction

Leishmaniasis is one of the deadliest neglected tropical diseases worldwide caused by intracellular hemoflagellate digenetic *Leishmania* species. This protozoan associated disorder is known to affect 12 million people globally, mostly in developing countries, and causing 30 000–50 000 mortalities annually. The disease is highly prevalent in tropical as well as subtropical regions such as Indian subcontinent and Southern Europe, and it has become a major health issue due to the lack of satisfactory treatment so far. The incidence of this disease is probably highly underestimated in most countries because of the lack of recognition and obligation. It is transmitted by the bite of infected female sandflies, *Phlebotomus* sp. which are the vectors of *Leishmania* parasites. The vector replicates as intracellular and aflagellated amastigotes in mammalian host[1]. In fact, off 600, only 60 sandflies are vectors for *Leishmania* around which 20 *Leishmania* sp. are associated with human pathogenicity. Leishmaniasis depends not only on the types of *Leishmania* sp., types of sandflies, and parasite genus but also number of bites, site of bites, and genetic potentiality[2]. Leishmaniasis occurs in three different clinical forms – cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis (VL). *Leishmania major* (*L. major*), *Leishmania tropica* (*L. tropica*), *Leishmania aethiopica*, *Leishmania mexicana* (*L. mexicana*), *Leishmania amazonensis*, and *Leishmania braziliensis* (*L. braziliensis*) are the major causative female parasites of CL. This dermal manifestation is known to affect sub-continent and Middle Eastern regions of the world. Although CL is non-lethal, it can cause psychological and social repercussion, stigmatization, painful disfiguration, severe secondary dermal manifestations neoplasms, and sarcoidosis. Lesions on face, nose, forehead, and lower limbs are the common symptoms of localized CL that usually heal naturally. On the other hand, nodules, lumps on the face, arms and legs are the symptoms of diffuse CL that never heal spontaneously[2]. In general, CL is known to affect the surface skin consisting mostly of ulcerated lesions, warty lesions or spots. Approximately 75% of CL is reported from Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Islamic Republic of Iran,

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North Sudan, Peru, and the Syrian Arab Republic[3,4].

In contrary to CL, mucocutaneous leishmaniasis is generally characterized by nasal obstruction and bleeding, disfiguration and generation of painful mucosal lesions, and cartilage of the mouth, ear and pharynx[2]. Particularly, mucocutaneous leishmaniasis is known to affect mucous membranes of the nose, laryngeal and pharynx.

Among three different clinical forms of leishmaniasis, visceral leishmaniasis (VL) is the most lethal form, representing a major public health problem in Indian subcontinent, East Africa, Mediterranean region and Latin America. VL is also termed as kala-azar that indicates a kind of systemic disease caused by *Leishmania donovani*, *Leishmania infantum (L. infantum*), and *Leishmania chagasi*. The irregular fever, weight loss, lack of appetite, hepatosplenomegaly, invasion of bone-marrow, skin pigmentation, and oral mucosa are the most common symptoms of kala-azar. In fact, the transmission of parasites in humans occurs from infected dogs or humans by the bite of sandflies. Kala-azar is the most severe form of the leishmaniasis and accounts for 200–400 thousands new cases and about 50 000 mortalities per annum[5]. *Leishmania donovani* causes anthroponotic transmission of kala-azar in Indian subcontinent as well as East Africa; while *L. infantum* is the causative agent of zoonotic transmission of kala-azar in the Mediterranean region, South America, and South-west and Central Asia[6]. In a nutshell, VL is known to affect bone marrow or internal organs, such as the liver, spleen and lymph nodes. Symptoms can also include anaemia, clotting problems, enlargement of the spleen, liver and lymph nodes.

The prevailing treatments for leishmaniasis caused severe side effects and emerged drug-resistant parasites, exhibiting major problems globally. From this point of view, there is an urgency to identify and design new, non-toxic, and inexpensive anti-leishmaniasis drug compounds from diversified anti-leishmaniasis agents. For these reasons, the complete genomes of *Leishmania* sp. have been decoded in order to investigate the proteins and mechanisms essential for the survival of the parasite. Certain *Leishmania* sp. proteins have been identified as potential targets through various computational tools.

2. Drug Search for Leishmaniasis (DSFL) database: Project overview

DSFL database is a unique part of World Community Grid that makes us available the best results for 50 crystallized proteins used during the project. These targeted proteins can be used as inputs for computational strategies in order to predict intermolecular interactions and relative binding energies to small organic molecules[7].

The identification of potential molecule candidates that could possibly be used in the designing and development of new efficacious drugs to combat leishmaniasis is the ultimate mission of DSFL. The extensive computing power of World Community Grid will be involved to execute computational study describing the interaction between several potent bioactive compounds from diverse sources and certain target proteins of *Leishmania* sp. The outcome will contribute towards the selection of the most active compounds that may lead to effective treatments for leishmaniasis. Table 1 enlists 50 crystallized target proteins along with their corresponding *Leishmania* sp., and expression system.

DSFL research team used AutoDock VINA software to search the ZINC database of commercially available effective compounds, best binding to selected target proteins of parasite. The database allows the selection of specific protein and provides the necessary information, including PDB link, protein's structure, data downloading in CSV format, VINA docking score of compound, 2D structure and ZINC database link.

The researchers involved in the DSFL project are Carlos Muskus (Principle investigator, PECET, University of Antioquia, Medellín, Colombia), Andres Florez (PECET, University of Antioquia, Medellín, Colombia), Rodrigo Ochoa (PECET, University of Antioquia, Medellín, Colombia), and Stan Watowich (The University of Texas Medical Branch, Galveston, Texas, USA). They identified protein drug targets by screening 6 00 000 molecules which might lead to the development of new drugs for this neglected and deadly disease. In fact, they searched for the most active drugs which target and bind to the protozoan proteins, resulting killing of *Leishmania* sp. Additionally, the investigation described the modification in the shape of proteins, which discusses few hurdles in identifying effective molecules. Further, *in vitro* study demonstrated the selection of one best drug candidate (3MJYcZ01) that was able to kill the parasite without affecting human cells[7].

3. DSFL database: A necessity in the current scenario

The classical treatments of leishmaniasis have serious side effects as well as high mortality rate which include the implementation of certain compounds such as sodium stibogluconate and meglumine antimoniate. Additionally, few other antileishmanial drugs *viz*. pentamidine and amphotericin B are not only very expensive and have severe side effects but also show difficulty in their mode of administration. Furthermore, the emergence of drug resistant parasites is the leading problem in several endemic countries. Currently, miltefosine (an oral antileishmanial drug) has been used successfully for the treatment of CL in Central and South America, and VL in India. But Phase IV clinical trial status of this drug in India has shown an enhancement in the relapse rate, indicating the possible development of drug resistance parasite in future. Surprisingly, there is no vaccine available against any form of leishmaniasis. The main reasons for the unavailability of vaccine against leishmaniasis are – (a) high cost of vaccine development and (b) lack of large enough market for pharmaceutical companies to invest a considerable amount of time and money to develop an effective vaccine. Therefore, a strong political will and considerable resources are required from the emerging market economies[58]. In view of the ongoing situation of leishmaniasis and lack of effective drugs for its treatment, we desperately need to test new bioactive molecules against target proteins of *Leishmania* sp. for beginning a new era in order to combat this disease.

The main mission of DSFL is to identify potential bioactive compounds or molecules from varied sources that could possibly be tested for the designing and development of new drugs against Leishmaniasis. The computational strategies of World Community Grid will be applied to understand the interactions between millions of bioactive compounds and certain target proteins of *Leishmania* sp. This will help to find the most promising and effective compounds that may lead to the possible treatments for this disease not only in a cost-effective manner but also without any side effects to mankind. The database helps to find unique target proteins of *Leishmania* sp. along with their necessary details. The structures of proteins of interest from different *Leishmania* sp. can be selected for molecular dynamics simulations.

Table 1
List of 50 crystallized target proteins of diverse *Leishmania* sp. for the development of new efficacious antileishmanial drugs.

Protein card	Protein name	Classification	Organism	Expression system	References
1E92	Pteridine reductase	Pteridine reductase	*L. major*	*E. coli*	[8]
1EVY	Glycerol-3-phosphate dehydrogenase	Oxidoreductase	*L. mexicana*	*E. coli*	[9]
1EZR	Nucleoside hydrolase	Hydrolase	*L. major*	*E. coli*	[10]
1IF2	Triosephosphate isomerise	Isomerise	*L. mexicana*	*E. coli* BL21	[11]
1LML	Leishmanolysin	Leishmanolysin	*L. major*	Not Available	[12]
1OKG	3-mercaptopyruvate	Transferase	*L. major*	*E. coli*	[13]
1Q50	Phosphoglucose isomerise	Isomerase	*L. mexicana*	*E. coli*	[14]
1R9J	Transketolase	Transferase	*L. mexicana*	*E. coli*	[15]
1SVV	Threonine aldolase	Lyase	*L. major*	*E. coli*	[16]
1TC5	tRNA deacylase putative	Structural genomics unknown function	*L. major*	*E. coli*	[17]
1X6O	Initiation factor 5a	Translation	*L. braziliensis*	*E. coli*	[18]
1XN4	Mar1 ribonuclease putative	Structural genomics unknown function	*L. major*	*E. coli*	[19]
1XTP	Sam-dependent methyltransferase	Structural genomics transferase	*L. major*	*E. coli*	[20]
1Y1X	Cell death 6 protein	Structural genomics unknown function	*L. major*	*E. coli*	[21]
1Y63	Kinase putative	Structural genomics unknown function	*L. major*	*E. coli*	[22]
1YF9	Ubiquitin conjugating enzyme e2	Structural genomics unknown function	*L. major*	*E. coli*	[23]
1YQF	Hypothetical protein 2	Structural genomics unknown function	*L. major*	*E. coli*	[24]
1Z2Q	Lm5-1 FYVE domain	Membrane protein	*L. major*	*E. coli* BL21 (DE3)	[25]
2A0U	EIF2	Translation	*L. major*	*E. coli*	[26]
2AR1	Hypothetical protein1	Structural genomics unknown function	*L. major*	*E. coli*	[27]
2B4W	Hypothetical protein 3	Structural genomics unknown function	*L. major*	*E. coli*	[28]
2C21	Glyoxalase I	Lyase	*L. major*	*E. coli*	[29]
2HFU	Mevalonate kinase	Transferase	*L. major*	*E. coli* BL21 (DE3)	[30]
2HQJ	Cyclophilin	Isomerase	*L. major*	*E. coli*	[31]
2I54	Phosphomannomu-tase	Isomerase	*L. mexicana*	*E. coli* BL21	[32]
2J63	Ap Endonuclease	Lyase	*L. major*	*E. coli*	[33]
2OEF	UDP-glucose pyrophosphorylase	Transferase	*L. major*	*E. coli*	[34]
2P18	Glyoxalase	Hydrolase	*L. infantum*	*E. coli*	[35]
2R8Q	PDEB1	Hydrolase	*L. major*	*E. coli* BL21	[36]
2VPS	Trypanothione synthase amidase	Ligase	*L. major*	*E. coli*	[37]
2W0H	Trypanothione reductase	Oxidoreductase	*L. infantum*	*E. coli*	[38]
2WSA	N-myristoyltransferase	Transferase	*L. major*	*E. coli*	[39]
2X77	Adp ribosylation factor-like 1	GTP Binding protein	*L. major*	*E. coli*	[40]
2XE4	Oligopeptidase B	Hydrolase	*L. major*	*E. coli*	[41]
2YAY	Dutpase	Hydrolase	*L. major*	*E. coli*	[42]
3CH7	6-phosphogluconolactonase	Hydrolase	*L. braziliensis*	*E. coli* BL21 (DE3)	[43]
3DWR	Coproporphyrinogen	Oxidoreductase	*L. major*	*E. coli*	[44]
3E3P	Glycogen synthase kinase	Transferase	*L. major*	*E. coli*	[45]
3FWU	Mif1	Cytokine	*L. major*	*E. coli*	[46]
3HA4	MIX Protein	Unknown function	*L. major*	*E. coli*	[47]
3IGZ	Phosphoglycerate mutase	Isomerise	*L. mexicana*	*E. coli* BL21	[48]
3KFL	Methionyl-tRNA synthetase	Ligase	*L. major*	*E. coli*	[49]
3KSV	Hypothetical protein 4	Unknown function	*L. major*	*E. coli*	[50]
3L4D	CYP51	Oxidoreductase	*L. infantum*	*E. coli*	[51]
3M3I	Hypothetical protein 5	Unknown function	*L. major*	*E. coli*	[52]
3MJY	Dihydroorotate dehydrogenase	Oxidoreductase	*L. major*	*E. coli*	[53]
3OH1	USP	Transferase	*L. major*	*E. coli*	[54]
3P01	Tyrosyl-tRNA synthetase	Ligase	*L. major*	*E. coli*	[55]
3PP7	Pyruvate kinase	Transferase	*L. mexicana*	*E. coli*	[56]
3Q5K	Hsp90 (amino-terminal)	Chaperone	*L. major*	*E. coli*	[57]

E. coli: *Escherichia coli*.

4. 'End Leishmaniasis' strategy through DSFL: How to achieve the goal?

Molecular docking will be performed using VINA software program from the Scripps Research Institute in La Jolla, California. The docking will be performed between the target protein of *Leishmania* sp. (selected from DSFL), and compound of interest obtained from various drug data bases. Further, the binding energy will be computed based on all possible orientation. If a compound or molecule binds to the target protein, it will disable the function of target protein and thus the multiplication of the parasite will be reduced, causing the prevention of progression of this tropical disease. In fact, DSFL will help to find the most promising compounds or molecules that may lead to 'End Leishmaniasis' strategy by targeting the specific protein of parasite. The computational study using DSFL database will be a significant replacement of expensive and time-consuming laboratory experiments.

5. Conclusions and future remarks

At present, the existing antileishmanial drugs, chemotherapeutic attempts, and immunotherapy options remain a dilemma to combat the leishmaniasis. The failure of antileishmanial drugs at clinical trial phase puts effort to identify new compounds from varied sources and to insight into the treatment of this parasitic disease by targeting the proteins of specific parasite. DSFL database provides the list of selected proteins of diverse *Leishmania* sp. that can be used for the development of new efficacious antileishmanial drugs. The effective bioactive compounds or molecules can be selected for designing and formulating new drugs after docking with specific target proteins available in DSFL database.

The information regarding unique target proteins of *Leishmania* sp. available in DSFL will be valuable for researchers to investigate new, non-toxic and inexpensive drugs for the treatment of leishmaniasis in future. Further, *in vitro/in vivo* study using the best compounds or molecules identified by inactivating the target proteins could lead to combat this neglected deadly tropical disease. In a nutshell, this database will help accelerate the ongoing research to begin 'End Leishmaniasis' strategy and to get rid of the era of drug resistance *Leishmania* sp. in future.

Conflict of interest statement

We declare that we have no conflict of interest.

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