Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Physics

Elixir Bio. Phys. 32 (2011) 1991-1994

ISSN: 2229-712X

In silico metabolic pathway analysis of Trichomonas vaginalis for potential drug targets

Singh.S[1,*], Singh.G[2], Gautam.B[1], Jain.P.A[1] and Yadav.P.K[1]

[1]Department of Computational Biology & Bioinformatics, JSBB, SHIATS, Allahabad-211007

[2]Department of Computer Science and Information Technology, CET, SHIATS, Allahabad-211007.

ARTICLE INFO

Article history:

Received: 28 January 2011;

Received in revised form:

21 February 2011;

Accepted: 1 March 2011;

Keywords

T. vaginalis,
In silico,
Drug targets,
Metabolic pathway,
KEGG,
Essential genes.

ABSTRACT

Trichomonas vaginalis causes the Trichomoniasis, in women and urethritis and prostate cancer in men. Presently Metronidazole, nitroimidazoles and tinidazole are used as the drugs of choice. But the emergence of drug resistant and cross resistance varieties of T. vaginalis has led to a search for novel drug targets. We have performed an insilico comparative analysis of metabolic pathways of the host Homo sapiens and the pathogen T. vaginalis. Enzymes from the biochemical pathways of T. vaginalis from the KEGG metabolic pathway database were compared with proteins from the host H. sapiens, by performing a BLASTp search against the non-redundant database restricted to the H. sapiens subset. The e-value threshold cutoff was set to 0.005. Enzymes, which do not show similarity to any of the host proteins, below this threshold, were filtered out as potential drug targets. Out of total 55 metabolic pathway in humans 26 metabolic pathways were identified when compared to the host H. sapiens. These 26 identified metabolic pathways contain 30 unique enzymes which are only present in T. vaginalis and absent in humans. The T. vaginalis genes for the identified enzymes were also retrieved for BLASTP search to identify any homologous protein in humans. Cysteine synthase and methionine gamma-lyase are two novel drug targets which were also reported well in literature and thus can be used to designing of inhibitors for the remedy of trichomoniasis.

Introduction

Recent advances in DNA sequencing technology have enabled elucidation of whole genome information from a plethora of organisms. To date, sequence information from large number of complete genomes has been deposited into various public domains . In parallel with the genome projects, a number of bioinformatics tools have been developed to facilitate in silico analysis of the gene sequence information [1]. The genome sequencing of T. vaginalis was carried out by The Institute of Genomic Research (TIGR) [2].

While drawing meaningful conclusions from a large amount of raw material, computer aided identification of suitable targets for further experimental analysis and characterization, has also led to the prediction of nonhuman homologous essential genes in Trichomonas vaginalis as promising candidates for novel drug discovery.

Here, we present a comparative metabolic pathway analysis to identify essential genes in *T. vaginalis*. Infection with *T. vaginalis* cause of trichomoniasis, number one nonviral and second most sexually transmitted disease (STD) [3, 4].

T. vaginalis was discovered in 1836 [5] and has been known to cause vaginitis since 1916, it was not until 1957 that an effective cure, metronidazole, was discovered.

But soon after drug resistance was first reported in 1962 [6]. Cross-resistance between different nitroimidazoles has been reported and is consistent with earlier studies.

Metronidazol is a prodrug, which need to be activated by enzymes before drug act on the desired target. In metronidazole-resistant *T. vaginalis*, the expression level of the hydrogenosomal enzymes pyruvateferredoxin reduced dramatically, which probably eliminates the ability of oxidoreductase, ferridoxin, malic enzyme, and hydrogenase are the parasite to activate metronidazole [7, 8, and 9]. The emergence of drug resistance in T. vaginalis is a major challenge in modern medicine. Therefore there is need to identify other novel drug targets so that new more effective drugs can be developed. In order to approach the defense against the protozoan parasite, differences in biochemical mechanisms between parasite and host are promising drug targets without expected cytotoxicity towards the human host.

Materials and methods

KEGG pathway database was used as a source of metabolic pathway information. Metabolic pathway identification names and numbers of the host *H. sapiens* and the pathogen *T. vaginalis* were extracted from the KEGG database [10]. Enzymes in these unique pathways as well as enzymes involved in other metabolic pathways under carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism were identified from the KEGG database (http://www.genome.jp/kegg/pathway.html). Only those enzymes were selected from the comparison of both metabolic pathways which are present in *T. vaginalis* and absent in *H. sapiens*. The corresponding protein sequences were retrieved from the KEGG database. They were subjected to a BLASTP [11] search against the non-redundant database with the *e*-value inclusion threshold set to 0.005

Tele:
E-mail addresses: satendralike@gmail.com

(http://blast.ncbi.nlm.nih.gov/Blast). The search was restricted to proteins from *H. sapiens* through an option available in the BLAST program, which allows the user to select the organism to which the search should be restricted. In the current context, the objective is to find only those targets, which do not have detectable human homologues. Enzymes, which do not have hits below the *e*-value inclusion threshold of 0.005, were picked out as potential drug targets.

Results and discussion

In the present study, Pathways which do not appear in the host but present in the pathogen according to KEGG database annotation have been identified as pathways unique to *T. vaginalis* as compared to the host *H. sapiens*. Out of total 55 metabolic pathways in *H. sapiens* only 26 were identified as common in both *H. sapiens* and *T. vaginalis.* While comparing these 26 different metabolic pathways we have identified 30 such unique enzymes which were only present in *T. vaginalis* and absent in *H. sapiens* via metabolic pathway comparison of pathogen and host approach (Table 1).

The corresponding gene and protein accession number of identified unique enzymes was retrieved from KEGG. These genes encode for proteins that include metabolic pathways under carbohydrate metabolism, amino acid metabolism, lipid metabolism, energy metabolism, vitamin and cofactor biosynthesis and nucleotide metabolism, surface structures, regulators, proteins involved in pathogenenicity, adaptation, information transfer, central/intermediate/miscellaneous metabolism pathways and some conserved hypothetical proteins of unknown function. Potential drug targets from the identified metabolic pathways could be useful for the discovery of new drugs molecules.

13 enzyme coding genes were identified which are involved in more than one metabolic pathway, for example gene TVAG_099490 is involved in fructose and mannose metabolism, starch and sucrose metabolism, all such enzyme coding genes with their corresponding metabolic pathways are listed in Table 2. Since most of these genes are involved in three to four different metabolic pathways, they appear more significant and essential for the survival of the *T. vaginalis.* Therefore these genes may provide new drug targets for the inhibitor designing.

For example, gene TVAG_387920, which encodes for Cysteine synthase and it appears to be involved in 3 metabolic pathways namely Cysteine and methionine metabolism, Selenoamino acid metabolism and Sulfur metabolism, represent an essential gene for *T. vaginalis* survival, may be probable novel drug target. *T. vaginalis* is an anaerobic protozoan parasite of humans and is rely heavily on cysteine as a major redox buffer, because it lacks glutathione. This has been reported that for synthesis of cysteine from sulfide, *T. vaginalis* relies upon cysteine synthase . As now it is also clear by metabolic pathway analysis that humans lack cysteine synthase; therefore, this parasite gene which encodes for enzyme Cysteine synthase could be an exploitable drug target [12,13]. Simillarly gene TVAG_147790 which encodes for enzyme methionine gamma-lyase can also be targated. The methionine gamma-lyase is a unique enzyme for sulfur-containing amino acid degradation [14]. Therefore this protein has also revealed potency for drug development [15].

Due to a lack of supporting data in the literature, about some of unique identified genes in *T. vaginalis*, these predicted genes reported in this paper are only a "first order guess" for probable novel drug targets. These identified genes can be annotated as putative proteins based on the functional relatedness of the BLAST data to other organisms. These genes are of particular interest for further characterization to verify the roles and essentiality for *T. vaginalis* survival. Thus, further investigations on these predicted genes are required to verify the prediction of these genes as novel drug targets.

Conclusion

The availability of full genome sequences and computer aided software to identify probable drug targets has become a new trend in genomics. Our *in silico* prediction has identified 30 essential genes, this approach has enabled rapid screening and identification of potential drug targets for further characterization in the laboratory.

T. vaginalis is a multidrug resistant pathogen and causes severe infection in humans. Novel active compounds targeted at these genes will be particularly useful in overcoming the detrimental consequence of *T. vaginalis* infection. The data presented here has identified new critical genes required for *T. vaginalis*. The number of essential genes is sufficiently small to allow for experimental analysis, leading to a systematic strategy in designing novel active compounds for the treatment *T. vaginalis* of infection. The metabolic pathway comparison of host and pathogen probably added more to our understanding of the *T.vaginalis.*

Acknowledgement

The authors are grateful to the Sam Higginbotom Institute of Agriculture, Technology & Sciences, Deemed University, Allahabad for providing the facilities and support to complete the present research work.

References

1. Kaminski, N. (2000). Bioinformatics. A user's perspective. Am. J. Respir. Cell Mol. Biol. 23, 705711.

2. Carlton JM, Hirt RP, Silva JC, et al. (2007) Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science (New York, N.Y.) 315 (5809): 207–12.

3. World Health Organization. (1995). An overview of selected curable sexually transmitted diseases, p. 2– 27. In Global program on AIDS, World Health Organization, Geneva, Switzerland.

4. Rein, M. F., and M. Mu¨ller. (1990). Trichomonas vaginalis and Sexually transmitted diseases 481–492. McGraw-Hill, New York, N.Y

5. Donne' A., (1836) Comptes rendus hebdomadaires des se'ances de l'Academie des Sciences Paris; 3:385.

6. Lossick, J. G. (1990). Therapy of urogenital trichomoniasis, p. 324–341. *In* B. M. Honigberg (ed.), Trichomonads parasitic in humans. Springer-Verlag, New York, N.Y.

7. Meingassner, J. G., and H. Mieth. (1976). Cross-resistance of trichomonads to 5-nitroimidazole-derivatives. Experientia 32:183–184.

8. Narcisi, E. M., and W. E. Secor. (1996). In vitro effect of tinidazole and furazolidone on metronidazole-resistant Trichomonas vaginalis. Antimicrob. Agents Chemother. 40:1121–1125.

9. Upcroft P. and Upcroft J.A. (2001). "Drug targets and mechanisms of resistance in the anaerobic protozoa". Clinical microbiology reviews 14 (1): 150–64.

10. Kanehisa, M., Goto, S., Kawashima, S., Nakaya, A., 2002. The KEGG databases at Genome Net. Nucleic Acids Res. 30 (1), 42–46.

11. Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new

generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.
12. Gareth D. Westrop, Gordon Goodall, Jeremy C. Mottram, and Graham H. Coombs(2006) Cysteine Biosynthesis in Trichomonas vaginalis Involves Cysteine Synthase Utilizing O-Phosphoserine , JBC Papers in Press,May 30, 2006, DOI 10.1074/jbc.M600688200
13. Singh S., Singh G., Gautam B., Varadwaj P., Farmer R., (2010) Trichomonas vaginalis genome analysis using bioinformatics approaches, International Journal of Pharmaceutical Sciences Review and Research, Volume 3, Issue 1, 38-42.
14. Nozaki, T.; Ali, V.; Tokoro, M. Sulfur-containing amino acid metabolism in parasitic protozoa. Adv. Parasitol., 2005, 60, 1-99.
15. Ali, V.; Nozaki, T. Current therapeutics, their problems, and sulfurcontaining- amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. Clin. Microbiol. Rev., 2007, 20(1), 164-187.

Table 1 Unique enzymes and their genes found in *T.vaginalis* metabolic pathway

S.No.	Metabolic Pathway	Enzyme	Enzyme Detail	T.vaginalis Gene ID
1	Pentose phosphate pathway	EC:2.7.1.12	Sugar kinase; gluconokinase	TVAG_135240
2	Fructose and mannose metabolism	EC:2.7.1.4	ROK family protein; fructokinase	TVAG_099490
3	Fatty acid biosynthesis	EC:1.3.1.-	oxidoreductase; enoyl-[acyl carrier protein] reductase II	TVAG_479680
4	Purine metabolism	EC:3.2.2.1and EC:2.7.2.2	Inosine-uridine preferring nucleoside hydrolase family protein; purine nucleosidase and Carbamate kinase; carbamate kinase	TVAG_213720 TVAG_261970
5	Alanine, aspartate and glutamate metabolism	EC:3.5.1.1 , EC:6.3.1.1and EC:1.4.1.4	Family T2; K01424 L-asparaginase , hypothetical protein; aspartate--ammonia ligase and glutamate dehydrogenase	TVAG_258340 TVAG_340510 TVAG_025910 TVAG_196700
6	Glycine, serine and threonine metabolism	EC:4.2.3.1, EC:4.1.2.5, EC:1.1.1.95 and EC:1.8.1.4	Threonine synthase family protein; threonine synthase , threonine aldolase; threonine aldolase , D-isomer specific 2-hydroxyacid dehydrogenase; K00058 D-3-phosphoglycerate dehydrogenase and dihydrolipoamide dehydrogenase family protein	TVAG_203590 TVAG_496760 TVAG_154750 TVAG_272760
7	Cysteine and methionine metabolism	EC:2.5.1.47, EC:3.2.2.9 and EC:4.4.1.11	Cysteine synthase; MTA/SAH nucleosidase family protein; S-adenosylhomocysteine/5'-methylthioadenosine nucleosidase and methionine gamma-lyase; methionine-gamma-lyase	TVAG_387920 TVAG_296300 TVAG_147790
8	Valine, leucine and isoleucine biosynthesis	EC:4.3.1.19	Pyridoxal-phosphate dependent enzyme family protein; threonine dehydratase	TVAG_090490 TVAG_362020
9	Lysine degradation	EC:3.4.-.-	Hypothetical protein ; Clan MP, family M67, Poh1-like metallopeptidase; COP9 signalosome complex subunit 5	TVAG_455170
10	Arginine and proline metabolism	EC:3.4.13.3 , EC:4.3.1.12 , EC:1.4.1.4 and EC:2.7.2.2	Clan MH; K01270 aminoacylhistidine dipeptidase , Ornithine cyclodeaminase/mu-crystallin family protein; ornithine cyclodeaminase , glutamate dehydrogenase; glutamate dehydrogenase (NADP+) and Carbamate kinase.	TVAG_056190 TVAG_090090 TVAG_385820 TVAG_419590 TVAG_025910 TVAG_196700 TVAG_261970
11	Histidine metabolism	EC:3.4.13.3	Clan MH; aminoacylhistidine dipeptidase	TVAG_056190 TVAG_090090 TVAG_385820
12	beta-Alanine metabolism	EC:3.4.13.3	Clan MH; aminoacylhistidine dipeptidase	TVAG_056190 TVAG_090090 TVAG_385820
13	Selenoamino acid metabolism	EC:4.4.1.11and EC:2.5.1.47	Methionine gamma-lyase; methionine-gamma-lyase and cysteine synthase	TVAG_147790 TVAG_387920
14	Cyanoamino acid metabolism	EC:3.5.1.1 and EC:6.3.1.1	Family T2; L-asparaginase and hypothetical protein; aspartate--ammonia ligase	TVAG_258340 TVAG_340510
15	Glutathione metabolism	EC:3.4.13.3	Clan MH; aminoacylhistidine dipeptidase	TVAG_056190 TVAG_090090 TVAG_385820
16	Starch and sucrose metabolism	EC:2.7.1.4 , EC:2.4.1.21 , EC:3.2.1.2, EC:3.2.1.3 and EC:2.4.1.25	ROK family protein , glycosyl transferase; starch synthase , glycosyl hydrolase; beta-amylase , hypothetical protein; glucoamylase and 4-alpha-glucanotransferase family protein; 4-alpha-glucanotransferase	TVAG_099490 TVAG_258220 TVAG_175670 TVAG_424960 TVAG_120280 TVAG_120430 TVAG_154680 TVAG_157940 TVAG_222040 TVAG_226870
17	Amino sugar and nucleotide sugar metabolism	EC:2.7.1.4	ROK family protein; fructokinase	TVAG_099490
18	Inositol phosphate metabolism	EC:4.6.1.13	Phosphatidylinositol-specific phospholipase C; 1-phosphatidylinositol phosphodiesterase.	TVAG_301070 TVAG_372910
19	Butanoate metabolism	EC:1.1.1.157	3-hydroxyacyl-CoA dehydrogenase; 3-hydroxybutyryl-CoA dehydrogenase	TVAG_333090
20	Vitamin B6 metabolism	EC:4.2.3.1	Threonine synthase family protein; threonine synthase	TVAG_203590
21	Nicotinate and nicotinamide metabolism	EC:3.2.2.1	Inosine-uridine preferring nucleoside hydrolase family protein; purine nucleosidase	TVAG_213720
22	Pantothenate and CoA biosynthesis	EC:1.1.1.169	Ketopantoate reductase PanE/ApbA family protein; K00077 2-dehydropantoate 2-reductase	TVAG_538040
23	Biotin metabolism	EC:2.6.1.62 , EC:2.8.1.6 and EC:3.4.-.-	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase family protein; adenosylmethionine-8-amino-7-oxononanoate aminotransferase , radical SAM domain containing protein; biotin synthetase and hypothetical protein	TVAG_258770 TVAG_388630 TVAG_455170
24	Lipoic acid metabolism	EC:2.7.7.63	Lipoyltransferase and lipoate-protein ligase containing protein; lipoate-protein ligase A	TVAG_094820
25	Nitrogen metabolism	EC:2.7.2.2 , EC:1.4.1.4 , EC:3.5.1.1 and EC:6.3.1.1	Carbamate kinase , glutamate dehydrogenase; glutamate dehydrogenase (NADP+) , L-asparaginase and hypothetical protein; aspartate--ammonia ligase	TVAG_261970 TVAG_025910 TVAG_196700 TVAG_258340 TVAG_340510
26	Sulfur metabolism	EC:2.5.1.47	Cysteine synthase	TVAG_387920

Table 2 Genes involved in more than one metabolic pathway

S.No.	T.vaginalis Gene	Metabolic Pathway
1.	TVAG_099490	1. Fructose and mannose metabolism 2. Starch and sucrose metabolism 3. Amino sugar and nucleotide sugar metabolism
2.	TVAG_261970	1. Purine metabolism 2. Arginine and proline metabolism 3. Nitrogen metabolism
3.	TVAG_258340	1. Alanine, aspartate and glutamate metabolism 2. Cyanoamino acid metabolism 3. Nitrogen metabolism 4. Biosynthesis of secondary metabolites
4.	TVAG_340510	1. Alanine, aspartate and glutamate metabolism 2. Cyanoamino acid metabolism 3. Nitrogen metabolism 4. Biosynthesis of secondary metabolites
5.	TVAG_025910	1. Alanine, aspartate and glutamate metabolism 2. Arginine and proline metabolism 3. Nitrogen metabolism
6.	TVAG_196700	1. Alanine, aspartate and glutamate metabolism 2. Arginine and proline metabolism 3. Nitrogen metabolism
7.	TVAG_203590	1. Glycine, serine and threonine metabolism 2. Vitamin B6 metabolism
8.	TVAG_387920	1. Cysteine and methionine metabolism 2. Selenoamino acid metabolism 3. Sulfur metabolism
9.	TVAG_147790	1. Cysteine and methionine metabolism 2. Selenoamino acid metabolism
10.	TVAG_455170	1. Lysine degradation 2. Biotin metabolism
11.	TVAG_056190	1. Arginine and proline metabolism 2. Histidine metabolism 3. beta-Alanine metabolism 4. Glutathione metabolism
12.	TVAG_090090	1. Arginine and proline metabolism 2. Histidine metabolism 3. beta-Alanine metabolism 4. Glutathione metabolism
13.	TVAG_385820	1. Arginine and proline metabolism 2. Histidine metabolism 3. beta-Alanine metabolism 4. Glutathione metabolism