ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 5, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

IN SILICO METABOLIC PATHWAY ANALYSIS AND DOCKING ANALYSIS OF *TREPONEMA PALLIDUM* SUBS. *PALLIDUM NICHOLS* FOR POTENTIAL DRUG TARGETS

RADHA MAHENDRAN*, SUGANYA JEYABASKER, SHARANYA MANOHARAN, ASTRAL FRANCIS, URJA SHAH

Department of Bioinformatics, School of Life Sciences, Vels University, Chennai - 600 117, Tamil Nadu, India.
Email: mahenradha@gmail.com

Received: 30 January 2016, Received and Accepted: 22 February 2017

ABSTRACT

Objective: Syphilis is a sexually transmitted infection caused by the spirochaete, *Treponema pallidum* subspecies *Pallidum nichols*. In this study, a comparative metabolic pathway analysis and molecular docking were performed to identify putative drug targets.

Methods: The biochemical pathways of *T. pallidum* subs. *P. nichols* and *Homo sapiens* were compared using kyoto encyclopedia of genes and genomes pathway(KEGG). The amino acid sequence of the selected enzymes were retrieved and Blastp was performed. Out of 9 enzymes, enolase was modeled using ModWeb, and the structure was validated using RAMPAGE. The active sites were identified using Metapocket 2.0 and further docked using AutoDock 4.2.

Results: The enzymes which were not similar to that of *H. sapiens* were filtered out as potential drug targets. A total of 9 enzymes were retrieved which were present only in *T. pallidum* subs. *P. nichols.* The structure obtained from Homology modeling was validated using RAMPAGE which showed 96% of the residues in the favorable regions and 3% of the residues in the allowed region. Since the result obtained from RAMPAGE showed structural reliability further active sites were predicted. The docking analysis results showed the interaction between enolase and doxycycline. The atom H42 displayed in green has interacted with OD1 (Asp 317) with a distance of 1.9 Å depicts the best interaction and the structures were visualized using PyMol.

Conclusion: Through this study, doxycycline which has antibacterial effect and a derivative of tetracycline could be one of the potential ligands against enolase.

Keywords: Syphilis, *Treponema pallidum*, Kyoto encyclopedia of genes and genomes, Blastp, Metabolic pathway, Homology modeling, Docking.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i5.17367

INTRODUCTION

Syphilis is a chronic sexually transmitted disease caused by *Treponema pallidum* subsp. *Pallidum nichols* [1]. This motile, gram-negative spirochaete, can be transmitted both sexually and from mother to child and can invade virtually any organ or structure in the human body [2,3]. It rapidly disseminates from a site of inoculation in the genital region to diverse organs where it can establish persistent, even lifelong infection [4,5]. Although the primary route of transmission is through sexual contact, it may also be transmitted from mother to fetus during pregnancy or at birth, resulting in congenital syphilis [6]. Syphilis can be present in at least one to four stages: Primary, secondary, latent, and tertiary. It performs very differently in metabolism compared with other bacterial pathogens [7]. Early (primary or secondary) syphilis is typically marked by ulcerative lesions that occur initially at the site of inoculation, followed several months later by widespread cutaneous, mucosal, and even systemic manifestations of the dissemination of the causal agent, *T. pallidum.* The ill effects of syphilis, however, go far beyond the disease's effect on individual infected persons. Early syphilis is associated with the infection of sexual partners and an increased risk of acquisition or transmission of human immunodeficiency virus [8]. A systemic, long-term infection that if untreated can damage the cardiovascular and nervous systems, ultimately leading to debilitation and death [9]. It has the genes encoding enzymes involved in glycolysis but lacks those related to the tricarboxylic acid cycle and the electron transport system [10]. Effective antibiotic treatment is a key component of syphilis control programs [11]. In the absence of a vaccine, syphilis control is largely dependent upon identification of infected individuals and treatment of these individuals and their contacts with antibiotics. Although penicillin treatment is still effective, clinically significant resistance to macrolides has emerged in *T. pallidum* [12]. Metabolic pathways of the host *Homo sapiens* and the bacterium *T. pallidum* subs. *P. nichols* were extracted from the kyoto encyclopedia of genes and genomes (KEGG) database [13]. KEGG is an effort to link genomic information with higher order functional information by computerizing current knowledge on cellular processes and by standardizing gene annotations [14,15].

METHODS

Metabolic pathway analysis

Metabolic pathways of *T. pallidum* subs. *P. nichols* were compared with that of *H. sapiens* pathways using the KEGG pathway database. A comparison was made between the unique pathways present only in the pathogen and not in the host. Each selected pathway was screened for the presence of pathogen-specific essential enzymes, and their sequences were retrieved. This method would predict and infer the biological functions and genomic sequences. The enzymes present only in the bacterium and not the host were selected, and the corresponding protein sequences were retrieved [16].

Identification of essential and non-homologous proteins

These sequences were subjected to BLASTp search against the non-redundant database. Enzymes which do not have hits below the e-value inclusion threshold of 0.005 were picked out as potential drug targets [14].

Homology modeling

Homology modeling for the enzyme enolase was done using ModWeb. ModWeb is a comparative modeling web server that is an integral module of MODBASE. ModWeb accepts one or more sequences in the FASTA format and calculates their models using MODPIPE based on the best available templates from the protein data bank (PDB). Alternatively, ModWeb also accepts a protein structure as input, calculates a profile for each identifiable sequence homolog in the UniProt database, followed by modeling these homologs based on detectable templates in the PDB as well as the user-provided structure. The results of ModWeb calculations are available to the users through the MODBASE interface [17,18].

Model validation

Ramachandran plot was used to check the reliability of the predicted 3D model. The accuracy of the predicted models was evaluated using the RAMPAGE server [19] and checked for the structural reliability [20].

Active site identification

Metalpocket 2.0 is a consensus method, in which the predicted pocket sites from eight methods, LIGSITE^{CS}, PASS, Q-SiteFinder, SURFNET, Fpocket, GHECOM, ConCavity, and POCASA, are combined to improve the prediction success rate. There are three steps in MetaPocket 2.0 procedure: Calling-based methods, generating meta-pocket sites, and mapping ligand-binding residues [21].

Ligand selection

The ligand doxycycline has antibacterial activity, and it was obtained from the literature Fig. 1.

Docking

Molecular docking protocols are widely used for predicting the binding affinities for a number of ligands. Molecular docking is an effective and competent tool playing an important and ever-increasing role in rational drug design. Docking is a computational procedure of searching for an appropriate ligand that fits both energetically and geometrically the protein's binding site [22].

AutoDock

Molecular docking experiment is performed using AutoDock 4.2. The AutoDock tools (ADT) is used to add partial charges using Gasteiger method and to arrange the polar hydrogens in the protein. The ligand is set to have flexible torsion angles at all rotatable bonds, while the protein is prepared as a rigid structure [20]. AutoDock is performed by evaluating energies for both the bound and unbound states. It also incorporates a new charge-based desolvation method that uses a typical set of atom types and charges [23].

AutoDock requires pre-calculated grid maps, one for each atom type, present in the ligand being docked as it stores the potential energy arising from the interaction with macromolecule [24]. Intermediary steps, such as PDBQT files for protein and ligands preparation and grid box creation were completed using graphical user interface program ADT. AutoDock saved the prepared file in PDBQT format. AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 110 × 110 × 110 xyz points. A scoring grid is calculated from the ligand structure to minimize the computation time. AutoDock 2.0 was employed for docking using protein and ligand information along with grid box properties in the configuration file. AutoDock 2.0 employs iterated local search global optimizer. During the docking procedure, both the protein and ligands were considered as rigid. The results less than 1.0 Å in positional root-mean-square deviation was clustered together and represented by the result with the most favorable free energy of binding. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis [25].

Visualization

The outputs were exported to pymol for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active sites [26].

RESULTS AND DISCUSSION

Enzymes as drug targets

Out of the total pathways, 25 pathways were found to be common in both *T. pallidum* subs. *P. nichols* and *H. sapiens*. While comparing the pathways, Methane metabolism and carbapenem metabolism were two unique metabolism pathways only present in *T. pallidum* and absent in *H. sapiens*. There were totally 9 enzymes, out of which 7 enzymes were corresponding to the methane metabolism and 2 enzymes to carbapenem metabolism. The list of genes are given in Table 1.

The aminoacid sequences corresponding to these enzymes were retrieved, and Blastp was performed. Out of these 9 enzymes, enolase had a sequence identity of 62% which was further modeled using ModWeb using PDB ID: 1ixyA as template. The homology modeling of the enzyme enolase is shown in Fig. 2.

The modeled structure was validated using RAMPAGE server which depicts 96% in the allowed region Fig. 3.

Evaluation of residues

The following residues were found in the allowed region:
Residue [157: ASP] (−120.26, 69.85), Residue [159: LYS] (−80.49, 17.41), Residue [201: SER] (−51.75, 162.16), Residue [211: ASP] (−140.49, 75.62), Residue [212: LEU] (−101.53, −67.39), Residue [233: ARG] (−66.84, −72.83), Residue [284: PRO] (−71.42, 85.43), Residue [296: ASP] (−69.36, 37.15), Residue [315: GLY] (−120.48, 98.58), Residue [316: ASP] (−81.12, −69.21), Residue [335: CYS] (66.18, 171.36), Residue [336: ASN] (−153.12, 39.48), Residue [337: SER] (−179.65, 144.52).

The number of residues in favored region was approximately 412 (96.0%) and the number of residues in allowed region was 13 (3.0%). The number of residues in outlier region was 4 (0.9%).

Since the favorable region was 96%, further active site identification done using Metapocket 2.0 is given below with the active sites marked in red.

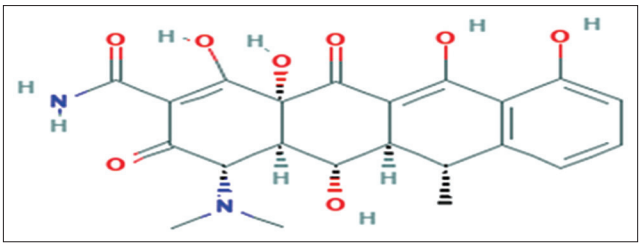

Fig. 1: Doxycycline (molecular formula: $C_{22}H_{24}N_2O_8$

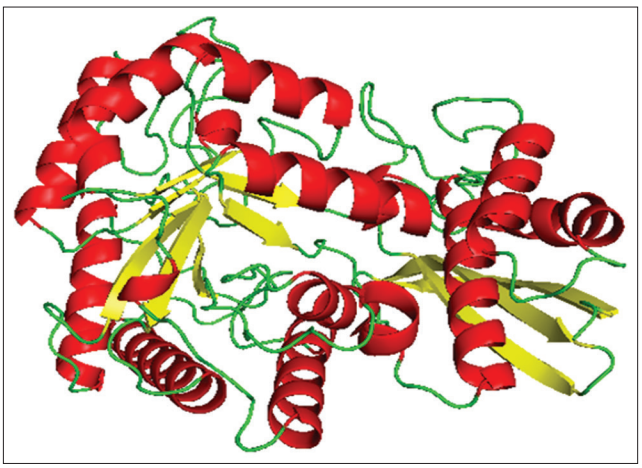

Fig. 2: Modeled structure of enolase (protein data bank ID): 1ixyA as template

Table 1: Metabolic pathways and genes present only in *T. pallidum* subs. *P. nichols*

Genes	Metabolic pathways	Enzyme/enzyme commission number
TP_0108	Methane metabolism	6-phosphofructokinase 1 [EC:2.7.1.11]
TP_0662	Methane metabolism	Fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
TP_0168	Methane metabolism	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [EC:5.4.2.11]
TP_0329	Methane metabolism	Glycine hydroxymethyltransferase [EC:2.1.2.1]
TP_0817	Methane metabolism	Enolase [EC:4.2.1.11]
TP_0094	Methane metabolism	Phosphate acetyltransferase [EC:2.3.1.8]
TP_0476	Methane metabolism	Acetate kinase [EC:2.7.2.1]
TP_0351	Carbapenem biosynthesis	Glutamate 5-kinase [EC:2.7.2.11]
TP_0350	Carbapenem biosynthesis	Glutamate-5-semialdehyde dehydrogenase [EC:1.2.1.41]

T. pallidum: Treponema pallidum, P. nichols: Pallidum nichols

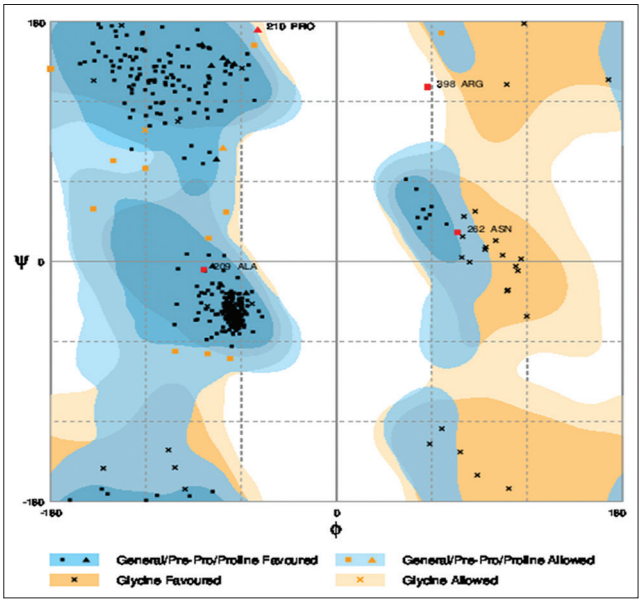

Fig. 3: Modeled protein structure evaluated by Ramachandran plot

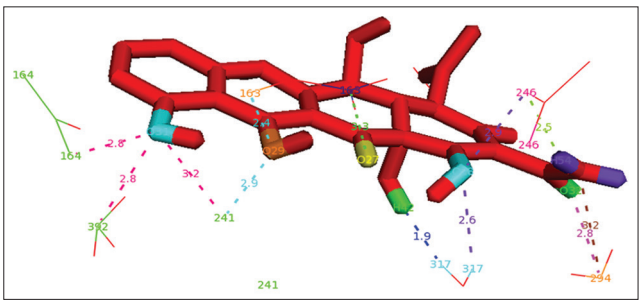

Fig. 4: Result of docking analysis showing interactions between the protein enolase and ligand doxycycline

MSDIACIEAREIIDSRGNPTVEVDVSLSDGSFGRACVPSGAST GEFEALEMR DGDKERYNGKGVLKAVGTVNTLIADTLEGMDALNQGEIDHAMRNL DGTDNKSKLGANAMLGVSMACARAAADFLGVPLYRYLGGVHTFRMPVP MANIINGGKHSDNKIDFQEFMVMPIGAASMREAVRMTAEVFHALKGLL AADGKATSVGDEGGFAPDLDNEQALEYIMKAIAKAGLAPRKDVCIA LDCASSELFDEGDRRGYKFWKSNPGKLFTAQEMIDLYKKWIATYPIV SIEDPLDQNDWAGYVQLTKELGDKVQIVGDDFFVTNTGRLARGIKE GSCNSILIKLNQIGTVTETVDAVRMAQNAGYAAVISHRSGETEDAFIADL AVALETGQIK TGSMSRSDRVAK YNQLMRIEEELGAQARYYGAKTFERFGC.

Docking analysis was done with the ligand doxycycline, and the interactions are shown in Fig. 4.

The atom O27 shown in yellow color has interacted with NE2 (Gln 163) with a distance of 3.3 Å, atom O28 shown in blue color has interacted with OE2 (Glu 246) with a distance of 2.9 and OD2 (Asp 317) with a distance of 2.6 Å, atom O29 shown in orange has interacted with OE1 (Gln 163) with a distance of 2.4 Å and OD2 (Asp 241) with a distance of 2.9 Å, atom O31 shown in blue color has interacted with OD2 (Asp 241) with a distance of 3.2 Å, NZ (Lys 392) with a distance of 2.8 Å and OE2 (Glu 164) with a distance of 2.8 Å, atom O32 shown in green color has interacted with NE2 (Gln 294) with a distance of 2.8 Å, atom H54 shown in purple has interacted with OE2 (Glu 246) with a distance of 2.5 Å, atom H42 shown in green has interacted with OD1 (Asp 317) with a distance of 1.9 Å. The atom H42 has the best interaction with a distance of 1.9 Å.

CONCLUSION

This approach has enabled rapid screening and identification of potential drug targets for further characterization. Novel active compounds targeted at these enzymes will be particularly useful in overcoming the detrimental consequences of *T. pallidum* subs. *P. nichols* infection. The data retrieved has identified new critical enzymes required for *T. pallidum*. The number of essential enzymes is sufficiently small to allow for experimental analysis, leading to a systematic strategy in designing novel active compounds for treatment of *T. pallidum* subs. *P. nichols* infection. The metabolic pathway comparison of the host and the bacteria probably added more to our understanding on *T. pallidum.* The enzyme Enolase had a sequence similarity which allowed to carry out homology modeling and docking analysis. Moreover, analysis of the docked ligand doxycycline with enzyme enolase has gained into focus of some important interactions operating at the molecular level.

ACKNOWLEDGMENT

We acknowledge Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing us with required infrastructure and support system needed.

REFERENCES

1. Liu J, Howell JK, Bradley SD, Zheng Y, Zhou ZH, Norris SJ. Cellular architecture of *Treponema pallidum*: Novel flagellum, periplasmic cone, and cell envelope as revealed by cryo electron tomography. J Mol Biol 2010;403(4):546-61.
2. Nyatsanza F, Tipple C. Syphilis: Presentations in general medicine. Clin Med (Lond) 2016;16(2):184-8.
3. Giacani L, Denisenko O, Tompa M, Centurion-Lara A. Identification of the *Treponema pallidum* Subsp. *Pallidum* TP0092 (RpoE) regulon and its implications for pathogen persistence in the host and syphilis pathogenesis. J Bacteriol 2013;195(4):896-907.
4. Radolf JD, Desrosiers DC. *Treponema pallidum*, the stealth pathogen, changes, but how? Mol Microbiol 2009;72(5):1081-6.
5. Houston S, Hof R, Francescutti T, Hawkes A, Boulanger MJ, Cameron CE. Bifunctional role of the *Treponema pallidum* extracellular matrix binding adhesin Tp0751. Infect Immun 2011;79(3):1386-98.
6. Olowe OA, Makanjuola OB, Olowe RA, Olaitan JO, Ojurongbe O, Fadiora SO. Prevalence of syphilis among pregnant women in two health care facilities in South Western Nigeria. Br J Med Med Res 2014;4(34):5431-8.
7. Chen X, Zhao M, Qu H. Cellular metabolic network analysis: Discovering important reactions in *Treponema pallidum*. Biomed Res

Int 2015;2015:328568.
8. Hook EW 3rd, Peeling RW. Syphilis control - A continuing challenge. N Engl J Med 2004;351(2):122-4.
9. Brinkman MB, McGill MA, Pettersson J, Rogers A, Matejková P, Smajs D, *et al.* A novel *Treponema pallidum* antigen, TP0136, is an outer membrane protein that binds human fibronectin. Infect Immun 2008;76(5):1848-57.
10. Ho EL, Lukehart SA. Syphilis: Using modern approaches to understand an old disease. J Clin Invest 2011;121(12):4584-92.
11. Centers for Disease Control and Prevention, Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. MMWR Recomm Rep 2006;55:1-94.
12. Stamm LV. Global challenge of antibiotic-resistant *Treponema pallidum*. Antimicrob Agents Chemother 2010;54(2):583-9.
13. Shobana K, Thiagarajan B. *In silico* identification of putative drug targets in *Neisseria meningitidis* through metabolic pathway analysis. SRJIS 2011;1(1):13-24.
14. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28(1):27-30.
15. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res 2010;38:D355-60.
16. Alves-Ferreira M, Guimarães AC, Capriles PV, Dardenne LE, Degrave WM. A new approach for potential drug target discovery through *in silico* metabolic pathway analysis using *Trypanosoma cruzi* genome information. Mem Inst Oswaldo Cruz 2009;104(8):1100-10.
17. Pieper U, Eswar N, Webb BM, Eramian D, Kelly L, Barkan DT, *et al.* MODBASE, a database of annotated comparative protein structure models and associated resources. Nucleic Acids Res 2009;37:D347-54.
18. Wei W, Minxuan X, Jie C, Fenni D, Rui Y, Xiaopei Z, *et al.* Genome-wide analysis of superoxide dismutase gene family in *Gossypium raimondii* and *G. arboretum*. Plant Gene 2016;6:18-29.
19. Zhang Z, Li Y, Lin B, Schroeder M, Huang B. Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction. Bioinformatics 2011;27(15):2083-8.
20. Krishnapriya MV, Sumathi T, Arulvasu C, Gopalsamy B, Renjith P. Comparative analysis of potentiality of esculin and hinokitol (β-thujaplicin) as anti-parkinsonism drugs: A pilot *in silico* study. Int J Pham Pharm Sci 2017;9(1):108-15.
21. Krivák R, Hoksza D. Improving protein-ligand binding site prediction accuracy by classification of inner pocket points using local features. J Cheminform 2015;7:12.
22. Azam SS, Abbasi SW. Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. Theor Biol Med Model 2013;10:63.
23. Vivitri DP, Adi Y. Structure-based design of novel rilpivirine analogues as HIV-1 nonnucleoside reverse transcriptase inhibitors through QSPR and molecular docking. IJPPS 2015;7(11):340-5.
24. Vijesh AM, Arun MI, Sandeep TT, Arulmoli T, Hoong-Kun F. Molecular docking studies of some new imidazole derivatives for antimicrobial properties. Arabian J Chem 2013;6(2):197-204.
25. Muhammad SA, Fatima N. *In silico* analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. Pharmacogn Mag 2015;11 Suppl 1:S123-6.
26. Anitha K, Gopi G, Senthil KP. Molecular docking study on dipeptidyl peptidase - 4 inhibitors. IJRDPL 2013;2(5):602-10.