International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319 – 6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 4; April 2017; Page No. 3060-3062
DOI: http://dx.doi.org/10.24327/ijcar.2017.3062.0181

Research Article

KEGG- A ROLE OF METABOLIC PATHWAY IN DRUG DESIGNING AGAINST *HELICOBACTER PYLORI* USING *IN SILICO* APPROACH

Manikandan. M., Sasidharan. P and Jayachitra. A*

Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, India

A R T I C L E I N F O

Article History:

Received 10th January, 2017
Received in revised form 19th February, 2017
Accepted 22nd March, 2017
Published online 28th April, 2017

Key words:

Helicobacter pylori, H.pylori, Protein Modeling, Swiss Model, Physicochemical Properties, Active Site, Ramachandran Plot, Kegg, Metabolic Pathway.

A B S T R A C T

Helicobacter pylori is a bacterium that infects half of the world's population and causes gastric disorders and severe diseases, including active chronic gastritis and gastric or duodenal ulcers. Peptidoglycan biosynthesis of *H.Pylori* reveals that protein with the entry HP0648 could be potential drug target. In this present study, we used different *In silico* tools and technique to analyze the protein sequence of HP0648 retrieved from the E.C 2.5.1.7. We predicted the 3-Dimenstional structure of protein sequence, its physiochemical properties and binding site port. 3D structure predicted by using the Swiss Model server and validated by Ramachandran plot analysis. We suggest that protein with the entry HP0648 could be a potential drug target for the *H.Pylori.*

INTRODUCTION

It has been known for more than a century that bacteria are present in the human stomach [1]. *Helicobacter pylori* is a gram-negative bacterium that infects half of the world's population and causes gastric disorders and severe diseases, including active chronic gastritis and gastric or duodenal ulcers. Moreover *H.Pylori* is considered a risk factor for the development of gastric cancer and mucosa-associated lymphoid tissue lymphoma (2). With increasing antibiotic resistance, *H.Pylori* is becoming more difficult to eradicate (3). The symptom of *H.Pylori* bacteria includes belching, bloating, nausea, vomiting and abdominal pain (4). As a world population started developing resistance to bacteria it is very important to discover a novel drug for the bacteria. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a knowledge base for systematic analysis of gene functions, linking genomic information with higher order functional information (5). Currently the best organized part of the KEGG database is metabolism. Each pathway can be viewed as a network of enzymes or a network of EC numbers (6). To identify the novel drug for the bacteria, it is efficient to identify the target pathway of the bacteria. The bacterial cell wall is often a target for antibiotic treatment. Cell wall consists of a layer called peptidoglycan, a molecule naturally found only in bacteria. Peptidoglycan layer act as a backbone of the cell wall.

Corresponding author:* **A. Jayachitra
Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, India

In this study we aim to target the backbone of the cell wall. Peptidoglycan biosynthesis of *H.Pylori* 26695 strain was analyzed by the KEGG database. (Fig 1). The pathway shows solved genes and unsolved genes as shown in the fig 1.

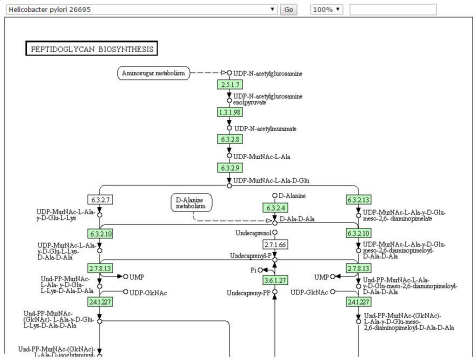

Fig1 KEGG- Peptidoglycan Biosynthesis of *H.Pylori* 26695

The EC number 2.5.1.7 has the protein and DNA sequence with entry (HP0648) and the protein sequence chosen as the target. HP0648 involves in the conversion of UDP-N-acetyl glucosamine to UDP-N-acetyl glucosamine enolpyruvate (Fig 2). By inhibiting this process at the initial level we can prevent the formation of peptidoglycan as end product. As a result of this inhibition, cell wall formation of the bacteria decreases in numbers and the growth gets stopped. UDP-N-acetyl glucosamine enolpyruvyl transferase catalyzes the first committed step in bacterial cell wall biosynthesis (7, 8). The enzyme transfers an enolpyruvyl group from

phosphoenolpyruvate (PEP) to UDP-*N*-acetyl glucosamine (UDPAG) to form UDP-*N*-acetyl glucosamine enolpyruvate.

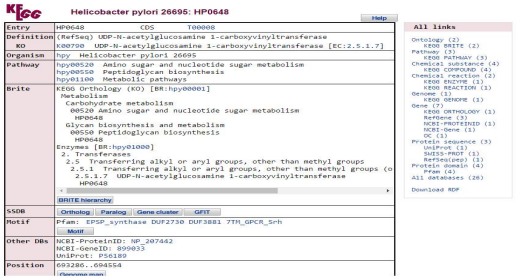

Fig 2 KEGG- *H.Pylori* 26695, Protein description

This is a precursor to UDP-*N*-acetylmuramate, an Essential building block for the bacterial cell wall. MurA is inhibited and because of its importance in peptidoglycan biosynthesis, it is of interest as a target for the design of novel antibacterial agents (9). In this present study, we analyze the protein sequence and predicted 3D structure of HP0648 protein from the peptidoglycan biosynthesis and also predicted the binding site of the 3D modeled protein. These all were analyzed by various Bioinformatics tools. These predictions can be further used for the drug designing purpose against *H.Pylori.*

MATERIALS AND METHODS

Selection of Target Pathway

Among 111 Metabolic pathway available for *H.Pylori* Peptidoglycan biosynthesis was chosen as the target pathway, since it is one of the vital component of the bacteria. This pathway is made up of 14 solved genes. The pathway starts with the conversion of UDP-N-acetyl glucosamine to UDP-N-acetyl glucosamine enolpyruvate

Selection of Drug Target

The Gene with the entry HP0648 falls at the initial level of the pathway was chosen as the target and it has a protein sequence with the length of 422 amino acid residues which doesn't have a structure in protein data bank (PDB). It's most efficient to inhibit the metabolism at the initial level.

Primary Structure Analysis of the Target

The Enzyme with the entry HP0648 has a Protein and DNA sequence. The protein sequence of the enzyme was retrieved. The physiochemical analysis were calculated for the protein sequence by ProtParam tool (http://web.expasy.org/ protparam/), which includes *pI*, total number of positively and negatively charged residues, the instability index (II), aliphatic index, and grand average of hydrophilic (GRAVY).

Structural Characterization of the Target

Secondary structure prediction was performed by using SOPMA (Geourjon and Deleage, 1995) server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pI?page=npsa_sopma. html). SOPMA is using homologue method of Levin *etal*. According to this method, short homologous sequence of amino acid will tend to form similar secondary structure.

Homology Modeling

The protein sequence was subjected for comparative homology modeling via Swiss model according to the method of Arnold [10] to generate putative 3D model. SWISS-MODEL is fully automated protein structure homology modeling server to make the protein modeling accessible to all Researcher. The SWISS MODEL performs the sequence alignments and searches for the putative template protein for generating the 3D model of the protein sequence.

Structure Validation using procheck

According to the method of Laskowski [11], PROCHECK checks the stereo chemical quality of a protein structure, producing a number of Post script plots analyzing its overall residue by residue geometry. It includes PROCHECK- NMR for checking the quality of structures solved by NMR. The structure was visualized and analyzed in Rasmol.

Binding Site Prediction

The binding site of the 3D Structure of protein was predicted by COACH server (http://zhanglab.ccmb.med.umich.edu/ COACH/). The binding site shows the predicted small pockets list where ligand bind to it.

RESULTS AND DISCUSSION

Primary structure analysis of the Target

The physiochemical analysis of HP0648 protein was performed using ProtParam and results were shown in Table 1.

Table 1 Physicochemical properties of HP0648 protein

Parameters	Values
Molecular weight	45657.16
Theoretical PI	8.16
Extinction Co-efficient	14690
Total number of negatively charged residues (Asp+Glu)	44
Total number of positively charged residues (Arg+Lys)	46
Instability index	34.53
Aliphatic index	112.89
GRAVY	0.081

HP0648 contains 422 amino acids with molecular weight 45657.16 Dalton. The total number of negatively charged residues (ASP + GLU) was 44 and the total number of positively charged residues (ARG + LYS) was 46. The isoelectric point pI was 8.16, protein is acidic in nature. The high aliphatic index (112.89) while instability index 34.53. The grand average of hydropathicity (GRAVY) is low 0.081.

Structural Characterization of the Target

The secondary structure of the protein was predicted using SOPMA server (Table 2).

Table 2 Secondary structure of HP0648 protein using SOPMA

Parameters	Values
Alpha helix	44.79%
Beta Sheets	19.67%
Coils	26.07%

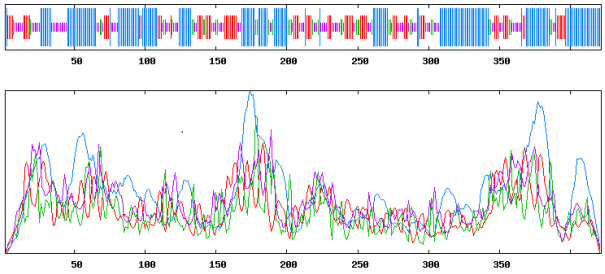

Fig 3 Secondary structure of HP0648 protein using SOPMA

It was observed that Alpha helix was predominant (44.57 %), followed by Random coil (26.07%) and beta sheet (19.67%). Alpha helix, in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of amino acid (Fig 3).

Homology Modeling and Validation

The Swiss-Model homology modeling program was used to predict the three dimensional structure of the Protein (HP0648) (Fig 4).

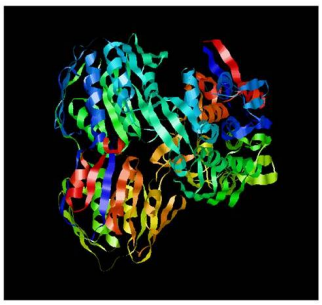

Fig4 Predicted 3D structure of HP0648 Protein using Swiss Model

PDB id 5UJS 2.45 Angstrom Resolution Crystal Structure of UDP-N-acetyl glucosamine 1-carboxyvinyltransferase from Campylobacter jejuni was selected as the template with 60% identity to the query sequence. The quality and validation of the model protein was evaluated by Ramachandran plot analysis using the PROCHECK server (Fig 5).

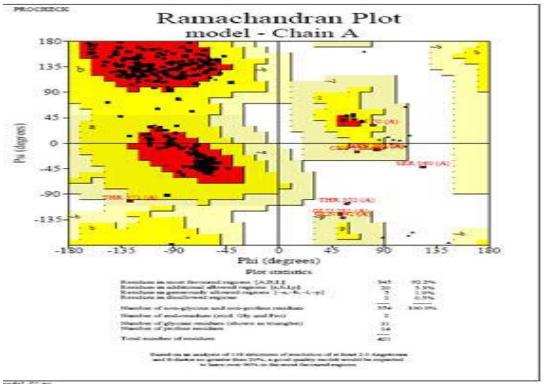

Fig 5 Ramachandran plot analysis

Ramachandran plot shows that Residues in most favored region 92.2 %, residues in allowed region 5.3% and residues in disallowed region 0.5%, it indicating that model is reliable and good quality.

Binding site prediction

The 3D structure of protein HP0648 was further analyzed for its binding site prediction using the COACH server.

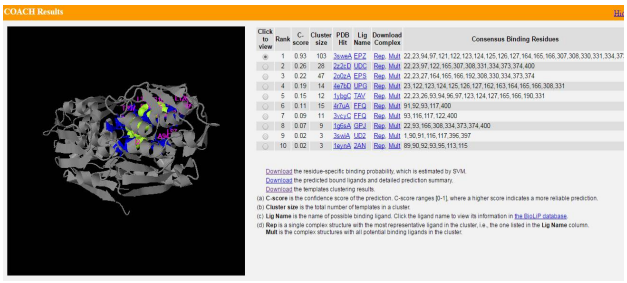

Fig 6 Binding site analysis of Predicted structure

This predicts that 3D structure of protein contains the list of small pockets were ligands bind to it. The possible binding site list as shown below (Fig 6).

CONCLUSION

The HP0648 protein involve in the conversion of UDP-N-acetyl glucosamine to UDP-N-acetyl glucosamine enolpyruvate. Inhibition of this process is much important to prevent the formation of peptidoglycan as end product. Peptidoglycan is one of the vital compounds of cell wall, further inhibiting the metabolism the bacteria growth decrease in high level. In this present study, we analyzed the physiochemical properties, Predicted 3D structure of the protein using Swiss-Model server. Further the predicted model was validated by using Ramachandran plot. Binding site of the protein was predicted by using the COACH server. From this we conclude that the protein with the entry HP0648 can be taken as target for the drug designing study towards *Helicobacter pylori*.

Reference

1. Bizzozero, G. 1893. Ueber die schlauchfo¨rmigen Dru¨sen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberfla¨chenepithel der Schleimhaut. Dritte mitteilung. Archiv Mikroskopische Anat. 43:82–152.
2. Malfertheiner P, Link A, Selgrad M. Helicobacter pylori: Perspectives and time trends. *Nat Rev Gastroenterol Hepatol* 2014; 11:628e38.
3. Testerman TL, Morris J. Beyond the stomach: an updated View of Helicobacter pylori pathogenesis, diagnosis and Treatment. *World J Gastroenterol* 2014; 20:12781e808. http://www.medicinenet.com/helicobacter_pylori/article.htm
4. Altan E, Masaoka T, Farre R, Tack J. Acotiamide, a novel gastroprokinetic for the treatment of patients with functional dyspepsia: postprandial distress syndrome. *Expert Rev Gastroenterol Hepatol*. 2012; 6(5):533-544. http://www.genome.jp/kegg
5. Ho¨ltje, J. V., and U. Schwarz. 1985. *In* N. Nanninga (ed.), Molecular cytology Of Escherichia *coli*, p. 77-109. Academic Press, New York, N.Y.
6. Rogers, H. J., H. R. Perkins, and J. B. Ward. 1980. Microbial cell walls and membranes. Chapman & Hall, London, England.
7. Kahan, F. M., J. S. Kahan, P. J. Cassidy, and H. Kropp. 1974. The mechanism of action of fosfomycin (phosphonomycin). *Ann. N. Y. Acad. Sci*. 235:364–385.
8. Kiefer, F, Arnald, K, Kunzli, M, Bordoli, L, Schwede, T, (2009), The SWISS-MODEL Repository and associated resources, Nucleic acids resources. 37:387-392
9. Laskowski, R. A**,** MacArthur, M. W**,** Moss, D. S**,** Thornton, J. M**, (**1993**),** PROCHECK: A Program to check the stereo chemical quality of protein structures**,** *J. Appl. Cryst*. 26:283-291.