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In Silico Sequence and Structural Annotation of Putative amidase in *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis (TB) is an infectious disease usually caused by the bacterium *Mycobacterium tuberculosis* (MTB). Tuberculosis generally affects the lungs, but can also affect other parts of the body. The classic symptoms of active TB are a chronic cough with blood-containing sputum, fever, night sweats, and weight loss. TB is spread from person to person through the air. TB can usually be cured and more than twenty drugs have been developed for treating TB. But most of the drugs were developed many years ago. The treatment usually consists of a combination of TB drugs that must be taken for at least six months. But the treatment will only be successful if the drugs are taken exactly as required for the entire length of time. If someone has drug resistant TB it means that the bacteria in their body won't be affected by certain drugs that they are resistant to. There is need for development of new drug for TB. Current study emphasized the finding novel role of putative amidase in the metabolism of bacteria in our body with its annotation. The present work focused on in silico sequence and structure analysis of this potential drug target involved in Mycobacterium tuberculosis by bioinformatics tools and database.
Keywords:- In Silico, amidase, annotation , Mycobacterium tuberculosis,etc

INTRODUCTION

Tuberculosis (TB) was one of the major global disease that leads to morbidity and mortality (1,2). Across the world one in three persons representing 2–3 billion individuals are known to be infected with *Mycobacterium Tuberculosis* (*M. Tuberculosis*) of which 5–15% are likely to develop active TB disease during their lifetime. In 2014, an estimated 9.6 million people fell ill because of the TB, around 1.5 million people died from the disease including 1.1 million HIV-negative persons and 400,000 HIV persons (3).

TB eradication efforts have included use of the most widely administered vaccine in human history, *Mycobacterium bovis* BCG. Besides this, TB continues to remain a constant source of human suffering, causing many failures on different levels in disease prevention, cure as well as health policy implementation. The protracted treatment period currently used for management of active TB disease is logistically complicated in resource-limited settings, which, together with other factors, has resulted in the rapid emergence of progressive drug-resistant TB (4)

This disease is an airborne bacterial infection caused by *M. Tuberculosis* which affects many parts of the body, most commonly the lungs (5). *M. Tuberculosis* is exposed to the air as droplet nuclei from coughing, sneezing, shouting of patients. Transmission occurs through inhalation of these droplet

nuclei which passes through the mouth or nasal cavities, the upper respiratory tract, bronchi then finally reaches the alveoli of the lungs (6).

Current study emphasized the finding novel role of putative amidase in the metabolism of bacteria in our body with its annotation. The present work focused on in silico sequence and structure analysis of this potential drug target involved in *Mycobacterium tuberculosis* by bioinformatics tools and database**.**

MATERIAL AND METHODS

1) Retrieval of Putative Amidase sequencefrom Protein database:- The Putative Amidase protein sequence from Mycobacterium tuberculosis was retrieved from Uniprot database. It is public protein database which contains the proteins amino acid sequences. The sequence was retrieved & saved in FASTA file format with its Accession ID(7).

2) Physicochemical analysis of Putative Amidase by Protparam tool:- Physicochemical properties of Putative Amidase were performed by using ProtParam analysis tool which on ExPASy server(8). It allows computation of various physical and chemical parameters for a given protein The computed parameters includes amino acid composition, molecular weight and theoretical pI, Instability index, Grand average of hydropathicity.

3) Identification of functional domain in Putative Amidase from Pfam database:-

Domain is the most important factor governing the protein folding into the structure. The domain of the Putative Amidase protein was predicted from the Pfam domain database which contains the information about protein families ,domains(9).

4) Secondary structure prediction and analysis of Putative Amidase:-

The secondary structure of Putative Amidase was predicted by SOPMA a secondary structure prediction method. SOPMA stands for self-optimized prediction method with alignment for the prediction of helix, strands and coils of the protein sequence(10).

5) Prediction, Validation & Visualization of 3D structure of Putative Amidase:- The 3D structure of Putative Amidase was predicted by using Swiss-model server. The selection of template was accomplished by protein BLAST using PDB database having identity more than 30%. The evaluation and validation of generated model was performed with PROCHECK server on PDBSum database and predicted model was visualized by Rasmol visualization tool .

RESULTS

i) Retrieval of amino acid sequence of Putative Amidase from protein database :

Putative Amidase [Uniprot ID: P9WQ97] sequence from *Mycobacterium tuberculosis was* retrieved from UniProtKB database with its 462 amino acids and saved in FASTA format which shown as below,

>sp|P9WQ97|AMIB2_MYCTU Putative amidase AmiB2 OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=amiB2 PE=1 SV=1
MDPTDLAFAGAAAQARMLADGALTAPMLLEVYLQRIERLDSHLRAYRVVQFDRARAEAEAA
QQRLDAGERLPLLGVPIAIKDDVDIAGEVTTYGSAGHGPAATSDAEVVRRLRAAGAVIIGKTNV
PELMIMPFTESLAFGATRNPWCLNRTPGGSSGGSAAAVAAGLAPVALGSDGGGSIRIPCTWCGL
FGLKPQRDRISLEPHDGAWQGLSVNGPIARSVMDAALLLDATTTVPGPEGEFVAAAARQPGRL
RIALSTRVPTPLPVRCGKQELAAVHQAGALLRDLGHDVVVRDPDYPASTYANYLPRFFRGISDD
ADAQAHPDRLEARTRAIARLGSFFSDRRMAALRAAEVVLSSRIQSIFDDVDVVVTPGAATGPS
RIGAYQRRGAVSTLLLVVQRVPYFQVWNLTGQPAAVVPWDFDGDGLPMSVQLVGRPYDEATL
LALAAQIESARPWAHRRPSVS

ii)Physicochemical analysis of Putative Amidase by Protparam tool:

Physiochemical properties of Putative amidase AmiB2 are shown in table 1.The instability index showed that it is stable. Very high aliphatic index of Putative amidase AmiB2 infers that it may be stable for wide range of temperature.

Sr.no	Parameters	Values
1	No. of amino acids	462
2	Molecular weight	49080.94
3	Theoretical pI	6.68
4	Instability index	34.99
5	Grand average of hydopathicity	0.001
6	No. of positively charged amino acids	43
7	No. of negatively charged amino acids	43

Table no.1:- Physicochemical Analysis

iii)Identification of functional domain in Putative Amidase from Pfam database:

The functional domain of Putative Amidase was predicted by Pfam database which shows single domain Amidase which is shown in Fig.no.1 and table no.2 depicts the start and end amino acid sequence.

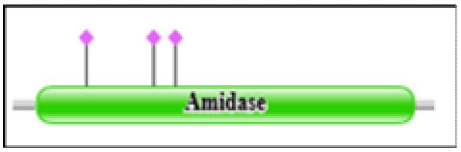

Fig.no.1:Domains predicted in Pfam database

Sr.no	Domain	Start	End
1	Amidase	27	442

Table .no.2: Starting and Ending of Amidase domain

vi) Secondary structure prediction and analysis of Putative Amidase:

The secondary structure of Putative Amidase was predicted by SOPMA Secondary Structure Prediction method. Secondary structural elements Alpha helices, strands & coils were enlisted in following Table no.3 and Fig no. 2. The table shows the Putative Amidase has more number of coils that is 42.62% followed by alpha helices 41.77% and strands 7.58%.

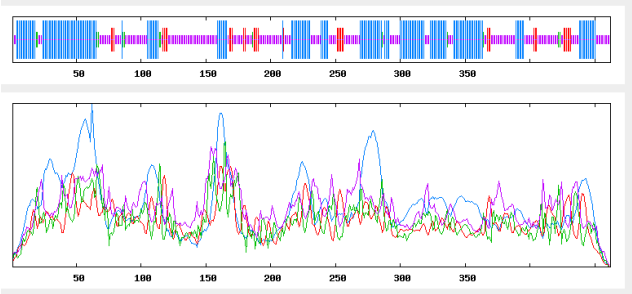

Fig.no.2: secondary structure of Putative Amidase using SOPMA

Sr.no	Secondary structures	No.of residues	Percentage
1	Alpha helices	193	41.77
2	Coils	35	7.58
3	Strands	220	42.62

Table no.3-Secondary structure Parmeters

v) Prediction, Validation & Visualization of 3D structure of Putative Amidase:

The homology modeling of Putative Amidase from *Mycobacterium tuberculosis* was obtained through SWISS MODEL server. The evaluation and validation of generated model were executed with PROCHECK server on PDBSum database which is shown in Fig.no.3.Validation of the predicted Putative Amidase from *Mycobacterium tuberculosis* by PROCHECK analysis showed that 92.5% of the residues of model were present in the most favoured region followed by 7.5% in the allowed region, 0.0% in generously allowed region and disallowed region respectively of Ramachandran plot which are shown in Fig. no.4 and Table no.3. Further the predicted structure was visualized by Rasmol viewer which is shown in Fig no.5.

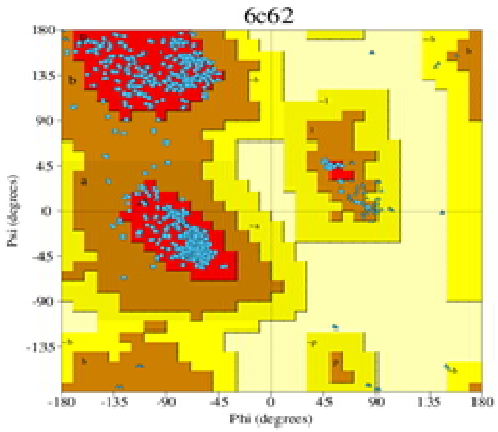

Fig.no.3: Ramchandran plot of Predicted 3D structure of Putative Amidase

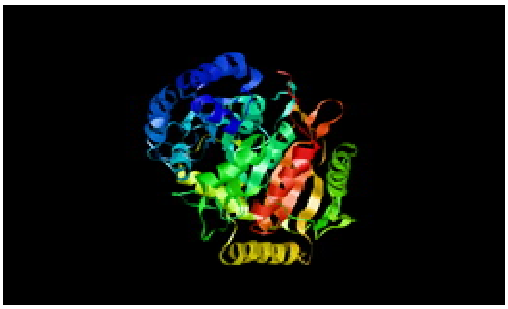

Figure 4: Predicted 3D structure of viewed in Putative Amidase RASMOL.

Sr.no	Regions	Residue no.	Percentage
1	Most favored regions [A,B,L]	792	92.5
2	Additional allowed regions[a, b, l, p]	64	7.5
3	Generously allowed regions [~a,~b, ~l, ~p]	00	0.0
4	Disallowed regions	00	0.0

Table no.3:- Ramchandran plot validation score

CONCLUSION

After studying role of Putative amidase in metabolism of Mycobacterium tuberculosis , it is estimated that it should be considered as target for drug designing for TB. It works as transporter protein which carries ammonium ions for different other metabolical reactions in *Mycobacterium tuberculosis*. Further the molecule is targeted by natural inhibotrs which lead to disturbed metabolism of pathogen which ultimately lead to death of it. Our further work is focused on *In silico* sequence and structure to find inhibitor Putative amidase involved in *Mycobacterium tuberculosis* by Computer Aided Drug Designing(CADD).

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