



## Molecular Modeling and Insilico Structural analysis of Mycobacterium Tuberculosis Mycosin 1

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### Abstract:

The Mycosins are subtilin like serine proteases present in Mycobacterium Tuberculosis. They have been reported to play a prominent role in Lysin degradation and Biotin metabolism. MycP-1 protein is one of the major proteins expressed in the patients infected with Mycobacterium Tuberculosis. The key metabolic process involved in Bacilli growth and survival is Biotin synthesis. Thus use of anti tubercular agents against the proteins involved in Biotin synthesis like Mycosins can combat the pathogenic effect caused due to Tuberculosis. The current work aims at constructing the protein model of MycP1 protein and interaction with its corresponding substrates. The Homology studies between various mycosin proteins and phylogenic analysis was carried out to determine the evolutionary strength among the proteins. To characterize the physicochemical and stereo chemical properties of MycP1 Functional and Structural analysis was performed. As the 3D structure determination of MycP1 was not reported. Using the Homology Modeling Approach, A protein model has been built. The model was developed using the Ab Initio Structure Prediction Method and further validated using Stereochemical analysis. Docking of substrates with Mtb-MycP1 has been performed to investigate the binding properties and energies of potent substrate of Mtb-MycP1 so as to propose effective and selective Mtb drugs for experimental validation.

**Key Words:** Mycosins, Mycobacterium tuberculosis, Ab initio structure prediction.

### Introduction:

Mycobacterium tuberculosis (M.tb) is the causative agent of tuberculosis (TB) and a successful pathogen that overcomes the numerous challenges presented by the immune system of the host. TB remains one of

the world's greatest causes of mortality and morbidity, with approximately 8 million new infections and 2 million deaths per year <sup>[1]</sup> More adults die due to TB every year than AIDS and Malaria together <sup>[2]</sup>. The Mtb genome revealed the presence of a large number of putative protease genes, some encoding potential secreted proteases <sup>[3]</sup>. This includes a family of five subtilisin –like serine proteases, the mycosins <sup>[4]</sup>, which share a high

Degree of similarity and are constitutively expressed in M. tuberculosis H37Rv <sup>[5]</sup>. The gene was found to be situated at 3700 bp (four ORF's) from the RD1 deletion region, which has attracted considerable attention recently because of possibly being a locus for potential diagnostic proteins. An observation of importance in homology modeling is that for a set of proteins that are hypothesized to be homologous, their three-dimensional structures are conserved to a greater extent when compared to their primary structures. This observation has been used to generate models of proteins from homologues with very low sequence similarities. Thus, in homology modeling, we are attempting to construct model of MycP1 from homologous proteins and analyzing the functional properties by performing Dockings.

### Materials and Methods:

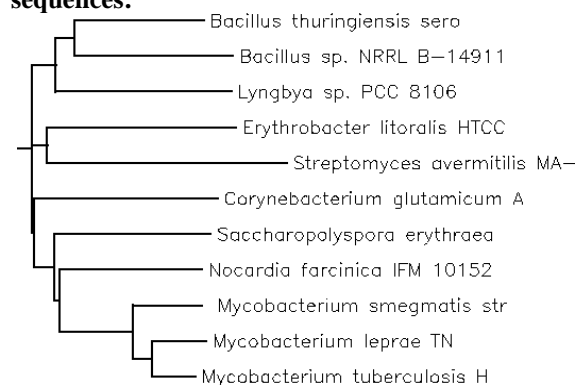
Mycosin Protein sequence was retrieved from the protein database of NCBI. Conservation studies were performed to find the conserved patterns which determine the homology strength of the protein in different mycosins. A phylogenetic tree was constructed to examine the evolutionary relationship between the bacterial homologs. Primary, Secondary and Tertiary structure analysis was performed to analyze the physico-chemical properties and to identify the structurally similar protein templates. For determining the 3D structure of Mycosin various Structural tools were used. The structural identity

was found to be below 35% with the various structural templates. Homology modeling approach was thus employed to build the protein structure. The method used is **Ab Initio Structure Prediction Method**. Among the models built, the best protein model was selected based on C Score. The obtained structure was further checked for Structure quality. Functional analysis was performed. The involvement of the mycosin in metabolic pathway was further analyzed by performing Binding Studies with its substrates, N6, N6, N6-Trimethyl-L-lysine and Biotinyl-L-lysine. The Docking studies were used to check the conformation and binding mode of substrate with Mycp1 which will indicate the essential aspects determining its binding affinity.

**Results and discussion:**

The Mycosin 1 protein sequence was retrieved from the protein database of NCBI. Conservation based on conserved patterns among the various Mycosin proteins of Mycobacterium Tuberculosis was analyzed. The patterns which show the homology strength between the mycosins are VAVIDTGV, GDGLSDCDAHGTVAS, IVAAAG, GPWV, GTSE, SGVAAALVR. The evolutionary distance between the homologs was predicted. The Phylogenetic analysis was performed to know the evolutionary significance of Mycosin protein in various bacterial species. From the dendrogram in Fig1 we can infer that the Mycosin from Mycobacterium Species are showing evolutionarily close relationship with Nocardia, Saccharopolyspora and Corynebacterium sps.

**Rooted Tree (dendrogram) for the selected sequences:**

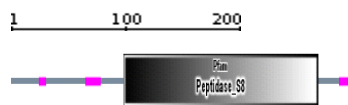


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**Fig. 1. Phylogenetic tree showing the divergence of MycP1 homologs.**

**Domain Analysis:**

From the domain analysis, the functional region of the Mycosin protein was identified as the **Peptidase\_S8 domain region** as shown in the Fig 2



**Fig 2: Domains within the Mycosin Protein sequence of 564 residues**

**Structure analysis:**

As per the Structural Analysis tools, the structural templates obtained showed less than 35% similarity with the Mycosin Protein. Using CPH model, HH Pred the PDB id 1DBI with 32.2% identity was found to show structure similarity with the Mycosin protein. By performing PDB Blast there was no structural template found with appreciable similarity for the given protein sequence. From the overall structural analysis and literature study it was confirmed that the structure of Mycosin protein could not be found in the data base. Thus the Homology modeling approach was used to model its structure. The method used to build the structure is Ab Initio Structure Prediction.

**Homology modeling:**

**Ab Initio Structure Prediction Method**

Based on the given protein sequence, this method aims to predict the protein structure in native conformation. 5 Model Structures were preposed. Based on the C Score (Confidentiality Score) Model 1 was selected to be the best.

**Ab Initio Structure Prediction Results:**

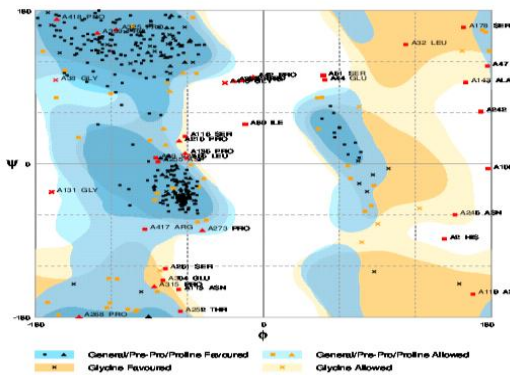
MODEL	C-SCORE
MODEL-1	C-score=-0.23
MODEL-2	C-score=-1.45
MODEL-3	C-score=-1.19
MODEL-4	C-score=-1.28

**Mycosin Protein Structure**



**Fig 3: the protein structure modeled using Ab Initio Structure Prediction**

**Ramachandran Plot:**



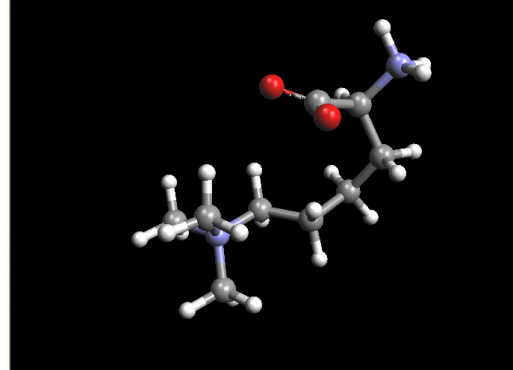
**Fig 4: Ramachandran plot**

From the above Ramachandran plot as shown in the Fig4 the quality of the Structure modeled was validated. Based on the presence of favored and allowed regions in the protein structure 81.1% of residues are laying in the favored region. This indicates that the protein modeled is structurally acceptable.

**Optimization of the selected Substrates/ Ligands:**

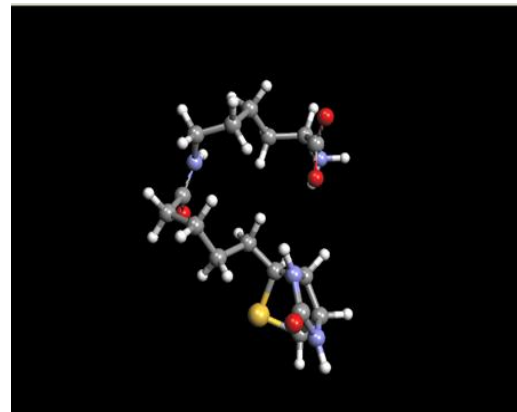
The substrates of Myosin involved in Biotin synthesis is N6, N6, N6-Trimethyl-L-lysine and Biotinyl-L-lysine. The chemical structures were retrieved and geometry optimization was performed.

**N6, N6, N6-Trimethyl-L-lysine:**



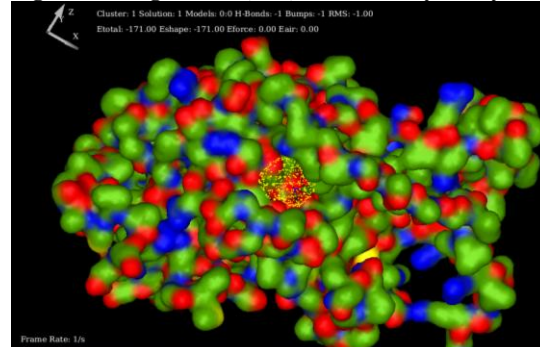
**Fig 5 showing the optimization energy for N6, N6, N6-Trimethyl-L-lysine is 31.15 kcal/mol**

**Fig 6: Biotinyl-L-lysine:**



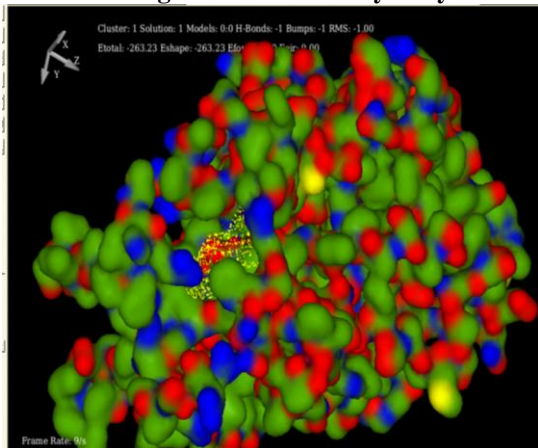
**The optimization energy for Biotinyl-L-lysine is 57.78kcal/mol**

**Fig 7: Docking with N6, N6, N6-Trimethyl-L-Lysine:**



The binding energy of Mycosin-1 of Mycobacterium Tuberculosis with N6,N6,N6-Trimethyl-L-Lysine is -171.00kcal/mole.

#### Docking with N6-D-Biotinyl-L-lysine:



The results for Mtb-MycP1 and substrates N6, N6, N6-Trimethyl-L-lysine, N6-D-Biotinyl-lysine shows best docked confirmations with lowest binding energy and the greatest number of confirmations per cluster.

#### Discussion:

An attempt was made in the present study to establish the Structural and Functional characterization of Mtb-MycP1 at molecular level using *insilico* analysis. The model built of Mtb-MycP1 using Ab Initio Structure prediction has the correct stereochemistry as gauged from the Ramachandran plot with a good three dimensional (3D) structure compatibility. Docking Studies was performed with suitable MycP1 substrates with MycP1 to analyze the substrate enzyme binding energies which will favor metabolic function of the protein. In du course of time, Knowledge of 3D structures of Target proteins plays a significant role in Molecular studies, Diversity analysis and Drug designing

#### Conclusion:

3D Protein Structure of MycP1 was modeled and is validated using ramachandran plot. Structural analysis was performed and the functionality of the protein was analyzed by docking with the substrates. The results showed the best docking confirmations

with the lowest binding energy and the greatest number of Conformations per cluster.

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#### References:

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