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Silico Functional Annotation and Molecular Characterization of an Uncharacterized Protein MBO_502153 of Mycobacterium Tuberculosis Variant Bovis

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ABSTRACT

Background and aim: Bovine, zoonotic, and wildlife tuberculosis are caused by Mycobacterium Bovis, whose attenuated form is used in tuberculosis and cancer treatment. There are several proteins present in its genome considered as uncharacterized or hypothetical proteins. We aim to predict the structure and function of an uncharacterized protein present in the bacterial genome of this pathogenic bacteria, Mycobacterium Bovis, by using several bioinformatics tools.

Materials and methods: Using in silico techniques, the secondary and tertiary structures of the selected uncharacterized proteins were predicted and further validated. Bioinformatics tools were used to explore physicochemical characteristics, homologous proteins, the active site, and the protein-protein interactions.

Results: By in silico study, we found that though the bacterium is a pathogenic microorganism, its uncharacterized protein MBO_502153 is non–virulent. This protein is a metabolism molecule. As the protein does not contain any kind of signal peptide, it is not a secretory protein. The uncharacterized protein MBO_502153 also does not involve any non-classical secretory pathway.

Conclusion: It can be concluded that the purposeful annotation of uncharacterized supermolecule showed some chemical activities, particularly degradation of the environmental waste product 4-chlorobenzoate and xenobiotics and synthesis of polyhydroxyalkanoate (PHA), which play an important role in environmental biodegradability and biocompatibility.

1. Introduction

A member of the Mycobacterium tuberculosis complex, Mycobacterium Bovis is a closely related taxonomic group of slowly growing (16-20 h generation time) mycobacteria. It is also a gram-positive, aerobic, and acid-fast bacteria.[1] For both humans and animals, M. Bovis shows a high level of virulence. One of the tuberculosis causative agents with a broad range of hosts includes cattle, humans, non-human primates, goats, cats, dogs, pigs, buffalo, badgers, possums, deer, and bison.[2] In developing countries, bovine tuberculosis also potentially represents a significant zoonosis as animals and humans share the same environment.[3] The Bovis strain's virulence was attenuated by successive passages on glycerinated bile–potatoes medium to create the Mycobacterium Bovis Bacille Calmette–Guérin (BCG) vaccine strain was first used as a tuberculosis vaccine against humans 1921.[4] The latest treatment for superficial bladder cancer is administering the bacterium Mycobacterium Bovis (BCG) via adjunctive therapy.[5] Next-Generation Sequencing (NGS) produces a greater quantity of genomic data from various bacterial organisms due to the latest technological advances. Almost half of the proteins belong to hypothetical or uncharacterized proteins in most genomes, and this protein class presumably has its significance for complete genomic and proteomic details.[6] Several proteins in a bacterium are thought to be hypothetical or uncharacterized proteins since their biochemical mechanisms and functions are still unclear. As a healthcare-associated bacterium, Mycobacterium Bovis has gained much interest in identifying novel characters in its genome in recent years. So, our study aimed to reveal the structural, physiological, and biological role of uncharacterized protein MBO 502153 of Mycobacterium Bovis for a depth study of this protein. In this study, we used the power of computational tools to annotate this hypothetical protein's potential functions, structure, and protein-protein interactions. The key organic contaminants are chlorinated aromatic compounds since they enter the atmosphere in vast numbers and are poisonous and resistant to degradation and bioaccumulation.[7] The biodegradation of polychlorinated biphenyls (PCBs), DDT, and some

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herbicides produces chlorobenzolate as dead-end metabolites.[8] In the biodegradation of such toxic chemicals in the ecosystem, microorganisms play a significant part. Many unknown pollutant-degrading microorganisms are present in polluted habitats playing a central role in bioremediation.[9] Conventional petrochemical plastics are microbial degradation recalcitrant causing environmental pollution. A class of water-insoluble polyester of various hydroxycarboxylic acids, PHAs are biodegradable thermoplastics that showed great potential to replace petroleum-based plastics.[10] Many bacteria produce eco-friendly biodegradable PHAs in intracellular granules inside their cells to store carbon and energy under unbalanced growth conditions such as depletion of nitrogen, phosphorous, or oxygen while an excess of a carbon source is still present.[11] Bags, containers, paper coatings, pens, golf tees, razors, feminine hygiene products, diaper back sheets, utensils, cosmetic containers, bottles, cups, and food packaging materials can all be produced with PHAs.[12] PHAs can be used as a surgical suture, skin substitutes, nerve cuffs, surgical meshes, implants, gauzes, staples, swabs, lubricating powders, blood vessels, tissue scaffolds, and bone fracture fixation plates, among other medical applications.[13] Researchers' efforts using diverse microbes have concentrated on the PHAs bioproduction.[14] This project seeks to use PHAs in bioreactors to manufacture industrially acceptable, environmentally friendly biopolymers.[15] However, PHA production costs are far greater than what would be necessary to keep up to compete on an industrial scale relative to standard non-biodegradable polymers.[12] By in silico approaches, we found that the uncharacterized protein MBO_502153 could degrade chloroaromatic compounds such as 4-chlorobenzoic acids and biosynthesize PHAs in this paper.

2. Materials and methods

Retrieval of uncharacterized protein sequence

The sequence of the uncharacterized protein MBO_502153 from Mycobacterium tuberculosis variant Bovis in the FASTA format was obtained from the NCBI website (https://www.ncbi.nlm.nih.gov). The complete layout that demonstrates all the tools used for structural and functional annotation of the protein is shown in Table 1.

Table 1. The complete layout demonstrates all the tools used for structural and functional annotation of the protein MBO_502153.

Purpose	Sequence Similarity	Physicochemical Characterization	Structure Analysis	Functional Annotation
Server name	BLASTp	ProtParam PSORTb CELLO PSLpred SOSUIGramN SOSUI TMHMM HMMTOP CCTOP Signal P 4.1 SecretomePv	Swiss model PSIPRED Endscript SOPMA PROCHECK Verify3D ERRAT SAVES SYMPRED	InterPro STRING VirulentPred PFP-FunDSeqE Pfam ScanProsite Superfamily CATH-Gene3D

Physicochemical properties

The physicochemical parameters of uncharacterized proteins were studied using Expasy's ProtParam server (www.web.expasy.org/protparam),[16] The molecular weight, theoretical pI, aliphatic index, amino acid composition, estimated half-life, atomic composition, extinction coefficient, the total number of positive and negative residues, instability index, and grand average of hydropathicity (GRAVY) were all measured using this method.[17]

Sub-cellular localization

A protein function is typically connected to its subcellular localization. It is widely known that cytoplasmic proteins can be used as drug targets, whereas surface membrane proteins can be used as vaccine targets.[18] The subcellular localization and topology of the uncharacterized proteins were predicted using CELLO (multi-class SVM classification system),[19] PSORTb,[20] PSLpred,[21] and SOSUIGramN,[22] TMHMM,[23] HMMTOP,[24] and CCTOP.[25] By SignalP server (http://www.cbs.dtu.dk/services/SignalP/), the signal peptide was estimated.[26] The role of uncharacterized protein in the non-classical secretory pathway was identified using (http://www.cbs.dtu.dk/services/SecretomeP/) SecretomeP.[27]

Function prediction

Similarity searches were conducted with the NCBI protein database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against non-redundant and the SwissProt[28] by using the BLASTp method to create a phylogenetic tree in order to predict the role of the targeted uncharacterized protein and to identify proteins that may have structural similarities with the uncharacterized protein. A conserved domain database (CDD) was searched on the NCBI website for a conserved domain to determine the uncharacterized protein's initial function. The search for protein motifs was conducted using the Motif server (https://www.genome.jp/tools/motif/). Pfam[29] and SuperFamily[30] database searches were performed to determine the protein's evolutionary relationships. The COILS server[31] was used for the identification of coiled-coil confirmation within the protein. The protein sequence analysis and classification server InterPro[32] were used for the functional analysis of the protein. PFP-FunD SeqE server[33] was used for protein folding pattern recognition. The STRING analysis was carried out to determine the potential functional network of protein interactions.

Virulence protein prediction and active site identification

In bacterial protein sequences, the recognition of virulent proteins helps estimate their pathogenic potential and recognize the complex virulence mechanism of pathogenesis,[34] for identifying the presence of virulence factor VirulentPred (http://bioinfo.icgeb.res.in/virulent/) and VCIMpred (http://crdd.osdd.net/raghava/vicmpred/submission.html) tools were used. Based on recent theoretical and algorithmic Computational Geometry, the Computed Atlas of Surface Topography of Protein (CASTp)

(http://sts.bioengr.uic.edu/castp/) was used to identify the active sites of the uncharacterized protein.

Secondary structure prediction

PSI-blast based on a simple and accurate 2D structure prediction server named PSIPRED server (https://bioinf.cs.ucl.ac.uk/psipred/), was used to obtain the probable 2D structure of the uncharacterized proteins. We also used the server SOPMA (https://npsa-prabi.ibcp.fr/cgibin/npsa_automat.pl?page=npsa_sopma.html) SYMPRED server (https://www.ibi.vu.nl/progr ams/sympr edwww/) and Endscript server (http://endscript.ibcp.fr/).

Tertiary structure prediction

The 3D prediction of the protein was made using SWISS-MODEL (https://swissmodel.expasy.org/) server by template-based modeling, HHpred server (https://toolkit.tuebingen.mpg.de/tools/hhpred) based on best scoring template, the automated protein structure prediction and Analysis tool Robetta (https://robetta.bakerlab.org/) Web server by continuous automated model evaluation and Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2) by advanced remote homology detection methods.

Quality assessment of 3D model

We used several quality assessment servers like- SAVES (https://servicesn.mbi.ucla.edu/SAVES/), PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/), Verify3D (http://nihserver.mbi.ucla.edu/Verify_3D/), and ERRAT Structure Evaluation server (https://servicesn.mbi.ucla.edu/ERRAT/ to identify the best predicted three-dimensional structure of the uncharacterized protein.

3. Results

Retrieval of uncharacterized protein sequence

The uncharacterized proteins MBO_502153, we selected from the organism Mycobacterium tuberculosis variant Bovis containing 311 amino acids. The source strain is Mycobacterium Bovis. The locus of the protein and accession is CEJ30992. Their version is CEJ30992.1.

Physicochemical properties

The uncharacterized protein MBO_502153 is 311 amino acids containing protein where the amino acids are Val (33), Ala (31), Pro (29), Gly (27), Asp (24), Ile (19), Arg (18), Leu (18), Ser (16), Glu (15), Gln (11), Lys (10), Met (10), Phe (9), Thr (9), Trp (9), Asn (8), His (7), Tyr (7) and Cys (1). So, the highest amino acid is valine (10.6%), and the lowest amino acid residue is cysteine (0.3%). The calculated molecular weight is 33971.75 Da, and the theoretical pI is 5.09. The total number of positively charged residues (Arg + Lys) is 28, and the total number of negatively charged residues (Asp + Glu) is 39. The instability index is 29.75. So, we can say that the uncharacterized protein is a stable one because having an instability index of less than 40. The aliphatic index is 87.14, giving an indication of protein stability over a wide temperature range. The GRAVY is -0.141. GRAVY with a negative value indicates that the uncharacterized protein is non-polar. The computed protein half-life is 30 hours in mammalian reticulocytes (in vitro), >20 hours in yeast (in vivo), and >10 hours in E. coli (in vivo). Protein has the molecular formula C1527 H2363 N417 O441 S11, which has a total of 4759 atoms.

Sub-cellular localization

Protein subcellular localization predicted from the CELLO, PSORTb, SOSUIGramN, and PSLpred server indicated that this protein is cytoplasmic. The value of Signal peptide, TAT signal peptide, Lipoprotein signal peptide, and other (the sequence does not have any signal peptide) from the result of SignalP-5.0 server are 0.0209, 0.003, 0.0064, and 0.9697, respectively. So, with the highest value of 0.9697, our uncharacterized protein is annotated as a protein that does not contain any signal peptide. Our uncharacterized protein is not a secretory protein because it receives a SecP score of 0.102965 from the SecretomeP 2.0a Server, lower than the recommended threshold of 0.5 for bacterial sequence. So, we can say that our uncharacterized protein does not involve in any non-classical secretory pathway. THMM, HMMTOP, and CCTOP servers predicted that no transmembrane helices present in the protein, and it is also not a transmembrane protein. THMM, HMMTOP, and CCTOP servers predicted that no transmembrane helices present in the protein, and it is also not a transmembrane protein.

Function prediction

The traditional method to annotate and explain the uncharacterized protein's function is to compare the protein's amino acid sequence with all the functional sequences of the databases. The result of BLASTp against the non-redundant Uniprot/SwissProt database is shown in Table-2. The outcome of the BLAST indicates that this protein functions as (R)-specific enoyl-CoA hydratases, which produce polyhydroxyalkanoate (PHA) synthesis from fatty acids. The existence of functional domains and hallmark motifs in uncharacterized proteins was investigated in relation to the biological role. Two domains: uncharacterized OB-fold protein-containing Zn-ribbon domain (accession No COG1545.) at 181-311, amino acid residues with an e-value of 1.74e-30, hot_dog superfamily (accession No. cl00509) at 26-160 amino acid residues with an e-value 6.10e-13 of the uncharacterized protein are found from the conserved domain search tool. Initially, the hotdog fold was found in some proteins with distinct catalytic activities like hydrolysis of 4-hydroxybenzoate-CoA, benzoyl-CoA, and the environmental pollutant 4-chlorobenzoate-CoA, phenylacetic acid degradation, xenobiotic degradation, fatty acid biosynthesis, and lipid metabolism.[35] The Pfam server represented the uncharacterized protein family of Mycobacterium Bovis has acyl-CoA-associated DUF35 OB-fold domain at 234-297 amino acid residues with an e-value of 3.10e-20 and MaoC dehydrate N (N-terminal half of MaoC dehydratase) at 18-160 amino acid residues with an e-value of 2.40e-16. A member of (R) enoyl-CoA hydratase group, MaoC, like hydratase involved PHA biosynthetic pathway from the β-oxidation cycle of fatty acids. The Superfamily server revealed the nucleic acid-binding proteins and Thioesterase/thiol ester dehydrase-isomerase superfamilies existence. The CATH-Gene3D server reveals the Hotdog Thioesterase protein superfamily. These results are also verified by using the InterProScan server. Usually, eight stranded α/β barrels containing '(TIM)-barrel' fold are found within the protein sequence by the PFP-FunDSeqE server. The STRING server revealed that our uncharacterized protein MBO_502153 is uncharacterized protein Rv3542c interacting with a conserved protein, an uncharacterized protein, three acyl-CoA dehydrogenases, three keto acyl-CoA thiolase, asteroid C26-monooxygenase, and asteroid 3-ketoacyl-CoA thiolase.

Table 2. The output of BLASTP against UniProt/Swissprot non-redundant sequences.

Organism Name	Accession	Max Score	Total Score	Query Cover	E-Value	Percent Identity	Accession Length
Mycobacterium tuberculosis complex	WP_003419293.1	629	629	100%	0.00	100%	311
Mycobacterium tuberculosis	WP_075911287.1	629	629	100%	0.00	99.68%	311
Mycobacterium tuberculosis	WP_086162714.1	629	629	100%	0.00	99.68%	311
Mycobacterium tuberculosis	WP_078429496.1	628	628	100%	0.00	99.68%	311
Mycobacterium canettii	WP_014001796.1	628	628	100%	0.00	99.68%	311

Virulence protein prediction and active site identification

From the VirulentPred tool, we identified that the protein is non-virulent. So, we can say that the protein is harmless and does not have any disease-producing power or ability. From the VICMpred result, the score of the uncharacterized protein in cellular process, information molecule, metabolism, and virulence factors are -0.02066812, -3.806919, 1.7992643, and -1.1322512, respectively, as well as the predicted functional class, is metabolism molecule. By protein folding, a groove or pocket is formed, which is called an active site. The amino acid residues present in the active site have various roles to play. They can participate in catalysis and binding of the substrate, help to stabilize the intermediates of the reaction or the binding cleft layout.[36] The Computed Atlas of Surface Topography of Protein (CASTp) revealed that 25 amino acids are involved in the effective active site of the uncharacterized protein (Fig. 1). The best active site has a surface area of 245.669 and a volume of 234.136 amino acids.

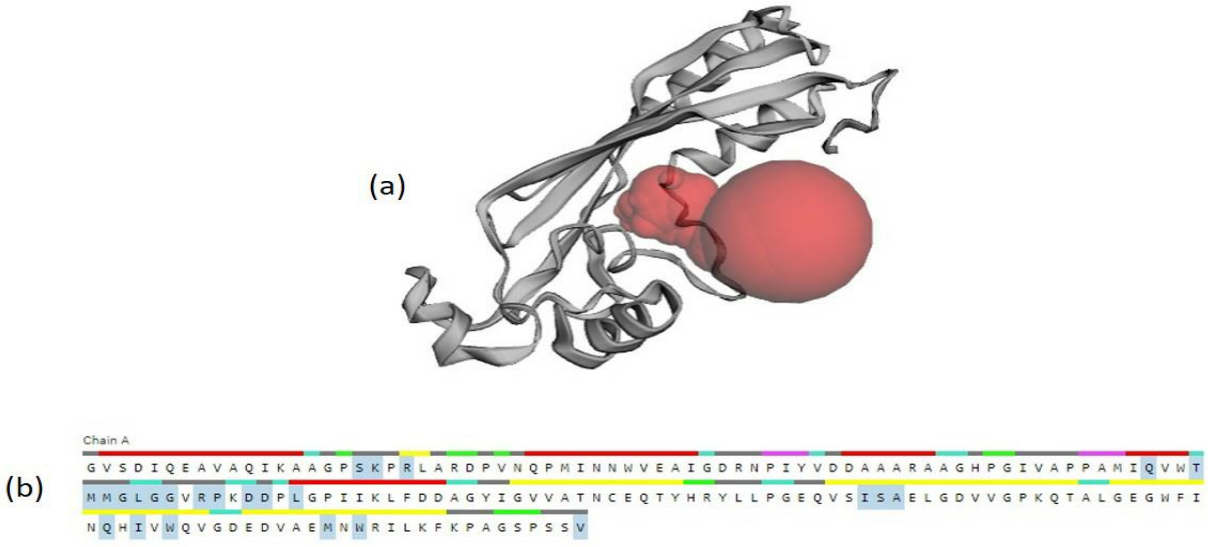

Fig. 1. The active site of the protein in red color(a) and amino acid residues of active sites in blue(b) of the uncharacterized protein MBO_502153.

Secondary structure

The SOPMA, SYMPRED, PRISPRED, and Endscript servers were used to find the secondary structure of the uncharacterized protein. From the result of the SOPMA server identified, the proportions of the random coil, the alpha helix, the extended strand, and the beta-turn of the uncharacterized protein are 49.20%, 26.05%, 19.29%, and 5.47%, respectively. Long coil regions, interrupted by short alpha-helices, extended strand, and beta-turn, appeared to be organized in the uncharacterized protein MBO 502153.

Tertiary structure

The protein MBO_502153 (Model-A) tertiary structure was obtained by template-based modeling using the SWISS-MODEL server. 1.50 Å crystallographic resolution of the highest template Hydratase ChsH1 protein (PDB id 4w78.2.A) with 100% identity was used to model the protein by following the X-ray diffraction method. The tertiary structure (Model-B) predicted from the HHpred server had 99.93% identity with the highest scoring (the template score was 161.23) template (PDB ID: 6OK1_B). Phyre2 server predicted tertiary structure (Model-C) with 100% identity with the highest template (PDB ID: c4w7bG). The predicted tertiary structure (Model-D) from the Robetta server provided high confidence 0.53 of the estimated model where 1.0 good and 0.0 poor. All four tertiary structures are given in Fig. 2.

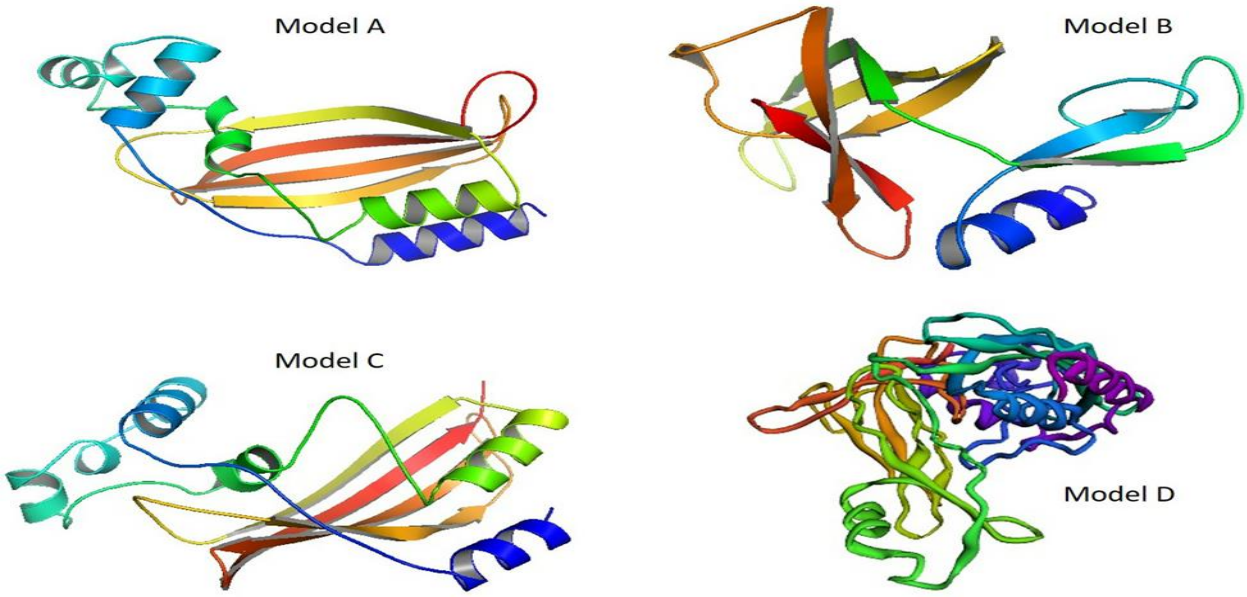

Fig. 2. The 3D structures Model A, Model B, Model C, and Model D of uncharacterized protein MBO_502153 predicted by Swiss Model, HHpred, Phyre2, and Robetta server.

The Swiss-Model Interactive Workplace also calculated the 3D model evaluation, which validated this model with a MolProbity score of 0.80 and a 97.09 percent accuracy. Ramachandran favored the Qualitative Model Energy Analysis (QMEAN), Global Model Quality Estimate (GMQE), Quaternary Structure Quality Estimation (QSQE), Cβ, all atoms, solvents and torsion values 0.62, 0.38, 0.53, 1.11, 0.95, -1.15 and 0.79 respectively. The Ramachandran plot assessment result from the SWISS-MODEL server of the selected desired protein is given in Fig. 3.

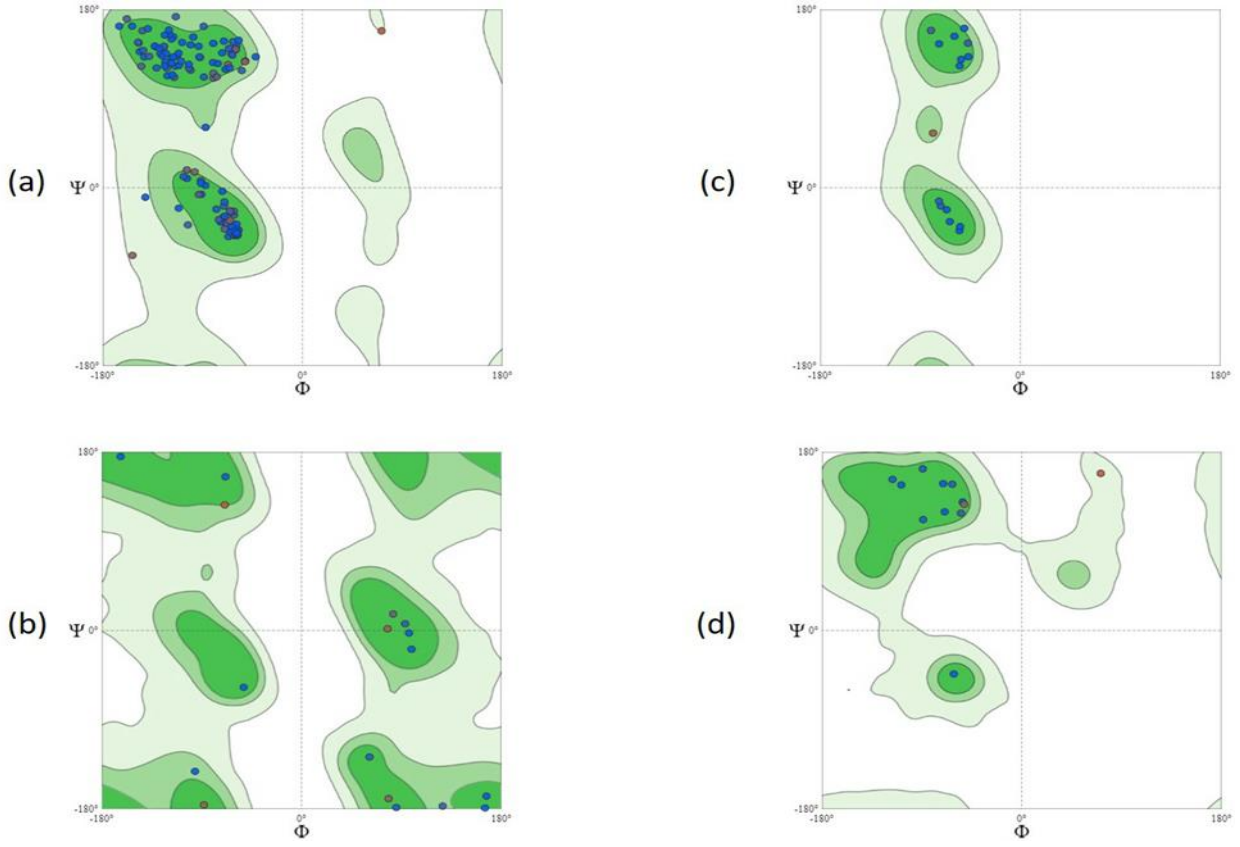

Fig. 3. The Ramachandran plot assessment (a) General, (b) Glycine, (c) Proline, and (d) Preproline.

4. Discussion

Mycobacterium Bovis causes tuberculosis (TB) which is an infectious disease. The infections can lead to significant economic loss for agricultural communities in livestock and other farmed animals in developing countries.[37] An uncharacterized protein MBO_502153 sequence in its genome was selected to analyze to understand this pathogenic bacterium in our research better. Using the SWISS-MODEL server to predict the proteins 3D model, the uncharacterized protein MBO_502153 showed the highest template identity of 100% with a PDB entry. This hypothetical protein is unique, and no similar template can be identified in any database. For structural evaluation of the four models, the SAVES server was used, which provides ERRAT, Verify3D, and Procheck server, and the results are shown in Table 3. Over 90% of residues in the most favored regions are expected in a high-quality model.[38] It is safe to expect that at least 80% of the amino acids in the 3D/1D profile will score >= 0.2. For good high-resolution structures, overall quality factor values of around 95% or higher are standard. The average overall quality factor for lower resolution structures is about 91%. By applying these parameters, the "best" model was chosen based on the highest assessment score. Model A's predicted tertiary structure of the protein MBO_502153 is the best of all the predicted structures (Fig. 2).

As the overall quality factor of Model-A is 98.718%, which is more than 95%, and 96.4 % of residues present in the most favored regions in the Ramachandran plot means more than 100% of residues present in the most favored regions, and 80% of amino acids scored >= 0.2 in the 3D/1D profile means more than % of amino acids have scored >= 0.2 in the 3D/1D profile, Model-A is our desired uncharacterized protein 3D structure with high quality. A maoC-like domain containing uncharacterized protein MBO_502153 is a member of the hotdog thioesterase family. This MaoC-like domain containing numerous bacterial proteins has (R)-specific hydratase enoyl-CoA function involved in the synthesis of polyhydroxyalkanoate (PHA).[39] The PHA's belong to a family of biologically degradable polyesters generated for the intracellular storage of carbon and energy by a wide range of microorganisms through the β-oxidation cycle. The hotdog fold is shared by a variety of proteins like beta-hydroxydecanoyl-acyl carrier protein (ACP)-dehydratase (FabA), 4-hydroxy benzoyl-CoA thioesterase (4HBT), fatty acid, and phospholipid biosynthesis regulator (FapR). Those seem to be unrelated. The FabA protein is a part of fatty acid degradation and lipid metabolism processes. The 4HBT protein hydrolyzes 4-hydroxybenzoate-CoA, benzoyl-CoA, and takes part in 4-chlorobenzoate degradation, which itself is a part of Xenobiotic degradation. The transcription factor FapR was DNA-binding and repressor, and biological functions were fatty acid biosynthesis, fatty acid metabolism, lipid biosynthesis, lipid metabolism, transcription, and transcription regulation. So, the Hotdog Thioesterase family proteins perform several functions like degradation of environmental pollutant 4-chlorobenzoate-CoA, phenylacetic acid, and xenobiotics, hydrolysis of 4-hydroxybenzoate-CoA and benzoyl-CoA, fatty acid biosynthesis as well as lipid metabolism.[35]

Table 3. The ERRAT, Verify3D, and Procheck server results compared 3D structures of Model A, Model B, Model C and Model D of uncharacterized protein MBO_502153.

Model	ERRAT (Overall quality factor)	Verify 3D (The percentage of residues that have averaged 3D-1D score >= 0.2)	Procheck (The percentage of residues in most favoured regions)
A	98.718	100.00%	96.4%
B	54.386	69.67%	93.0%
C	92.258	100.00%	93.2%
D	77.193	87.46%	88.1%

5. Conclusion

A detailed characterization of uncharacterized proteins is required to help researchers search for new proteins of interest To understand various features of proteins' unique structures. Computational approaches for understanding uncharacterized protein functions provide biologists with the ability to create a full record of their biological functions. To find Mycobacterium Bovis bacteria's other novel functions except healthcare use, we studied its uncharacterized protein MBO_502153. We have tabled and discussed many in silico methods for the functional predictions of uncharacterized protein from amino acid sequence to the structural stage like secondary and tertiary structure prediction, domain and motif recognition, comparative analysis, phylogenetic profiling, and more. Our current study found that our uncharacterized protein MBO_502153 makes bacteria capable of degrading the environmental waste product 4-chlorobenzoate. Our uncharacterized protein MBO_502153 aids bacteria in the production of the biodegradable thermoplastic polyhydroxyalkanoates. As the production cost of PHAs is too expensive to produce them commercially in industries. This suggests selecting and developing unique bacterial strains that can absorb or degrade diverse substrates effectively to turn them into PHAs with high quality and productiveness, as well as develop high-performance fermentation and effective extraction and purification process to a reduced the level of cost.

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