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Computational approaches for structure-based characterization and functional elucidation of a protein from *Acinetobacter baumannii* involved in siroheme biosynthesis

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INTRODUCTION & AIM

One of the main causes of hospital-acquired infections is the Gram-negative, exclusively aerobic bacterium Acinetobacter baumannii. It is classified as oxidase-negative and catalase-positive, and the majority of clinical cases are caused by two globally dominant clones.

Immunocompromised patients, including as the elderly, children, intensive care unit (ICU)/post-operative patients, and those needing continuous ventilation, are the main targets of this opportunistic bacterium. Numerous illnesses, including pneumonia, bacteremia, meningitis, urinary tract infections, wound infections, and ventilator-associated pneumonia, are linked to it.

Because of its remarkable capacity to develop multidrug resistance (MDR), especially to carbapenems, which are frequently considered the final resort for therapy, A. baumannii represents a serious threat to public health. Plasmids, transposons, and integrons play a major role in mediating resistance, which makes treatment choices more difficult and raises morbidity and mortality.

There are still unanswered questions about the pathophysiology, virulence factors, and transmission dynamics of A. baumannii despite a wealth of study on resistance mechanisms. The identification of elements essential to persistence and virulence, such as motility, adhesion, biofilm formation, iron acquisition, and important proteins like OmpA, phospholipases, PBPs, polysaccharides, and outer membrane vesicles, has been made easier by recent developments in whole-genome sequencing, molecular manipulation, and infection models.

METHOD

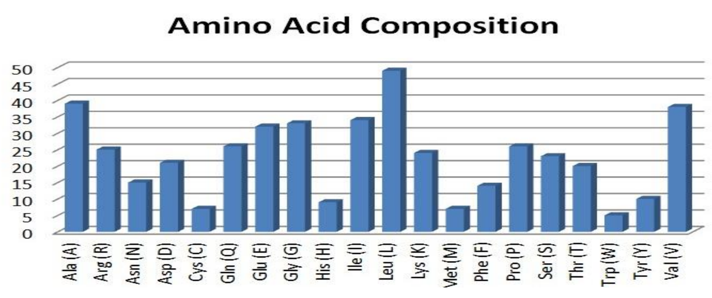
- ✓ Sequence Retrieval: Acinetobacter baumannii's protein sequence was obtained from UniProt in FASTA format.
- ✓ Physicochemical Characterization: Molecular weight, isoelectric point (pI), instability index, aliphatic index, and hydropathicity were all determined by analysis using ProtParam.
- ✓ Functional Annotation: NCBI CD-Search and ScanProsite were used to find conserved domains and functional motifs.
- ✓ Structure Prediction & Validation: SOPMA and PSIPRED were used to estimate the secondary structure.SWISS-MODEL, I-TASSER, and AlphaFold were used to model the tertiary structure.Models were checked for structural accuracy using ProSA-web and the UCLA-SAVES server.

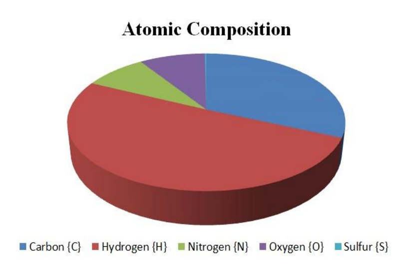
RESULTS & DISCUSSION

Sequence Retrieval and Physicochemical properties determination:

Acinetobacter baumannii's protein (UniProt: A0A219CBP8) has a molecular weight of 50.45 kDa, a pI of 6.19, and 457 amino acids. There are 49 positively and 53 negatively charged residues in it.

According to physicochemical examination, the protein is hydrophilic (GRAVY -0.065), thermostable (aliphatic index 103.48), and stable (instability index 39.34). For mammalian reticulocytes, the half-life is approximately 30 hours; for yeast, it is over 20 hours; and for E. coli, it is over 10 hours.





Functional annotation

The siroheme synthase CysG domain, which spans residues 1–456 in the protein, is a multifunctional enzyme that catalyzes the oxidation, methylation, and iron insertion into uroporphyrinogen III phases of siroheme production, according to analysis. Important anticipated responses consist of:

1) 2 H^+ + siroheme = Fe^{2+} + sirohydrochlorin
2) 2 S-adenosyl-L-methionine + uroporphyrinogen III = H^+ + precorrin-2 +2 S-adenosyl-L-homocysteine
3) NAD^++ precorrin-2 = 2 H^+ + NADH + sirohydrochlorin.

The protein is linked to several molecular processes and biological functions, according to Gene Ontology (GO) analysis:

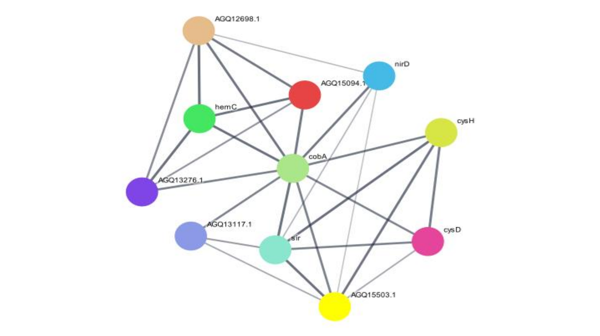
1. Molecular Functions: uroporphyrin-III C-methyltransferase, sirohydrochlorin ferrochelatase, precorrin-2 dehydrogenase, and NAD binding
2. Biochemical Processes: Biosynthesis of Cobalamin, Methylation, and Siroheme

Table 1. Gene Ontology Analysis

Accession	Name	Ontology
GO:0051288	NAD binding	Molecular Function
GO:0043115	precorrin-2 dehydrogenase activity	Molecular Function
GO:0051266	sirohydrochlorin ferrochelatase activity	Molecular Function
GO:0004851	uroporphyrin-III C-methyltransferase activity	Molecular Function
GO:0009236	cobalamin biosynthetic process	Biological Process
GO:0032259	methylation	Biological Process
GO:0019354	siroheme biosynthetic process	Biological Process

Moreover, the ScanProsite tool documented two motifs in the protein sequence namely Uroporphyrin-III C-methyltransferase signature-1(accession: PS00839, position: 221-235) and Uroporphyrin-III C-methyltransferase signature-2 (accession: PS00840, position: 296-329.

The PPI network of the selected protein was demonstrated by the string database (v.12.0) indicating that the protein is associated with 10 more proteins

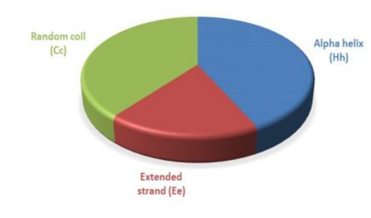


The PPI network

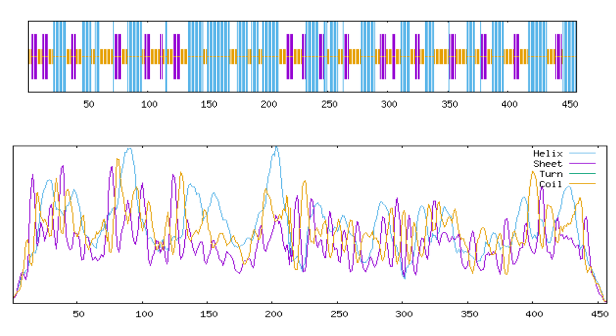
Structure prediction and validation

Importance of secondary structure:

Regular motifs known as secondary structures serve as the building blocks for a protein's three-dimensional folding. The SOPMA tool was used to forecast the presence of random coils, extended β-strands, and α-helices in the chosen protein. Hydrogen bonds stabilize these components, which are essential for healthy protein folding and function. The overall protein shape can be upset by mistakes in secondary structure formation, which may impact biological activity and exacerbate disease.



The secondary structural elements

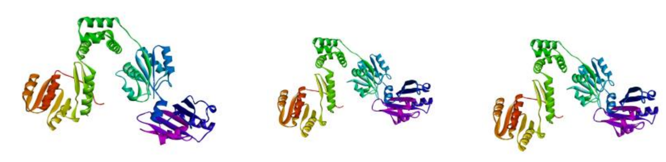


the secondary structural features are represented graphically

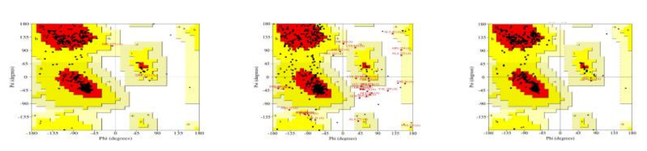
Importance of tertiary structure:

The interactions between amino acid residues, such as side chains and backbone atoms, determine the protein's distinct three-dimensional folding, which is represented by the tertiary structure. Protein stability and appropriate function depend on this level of structure.

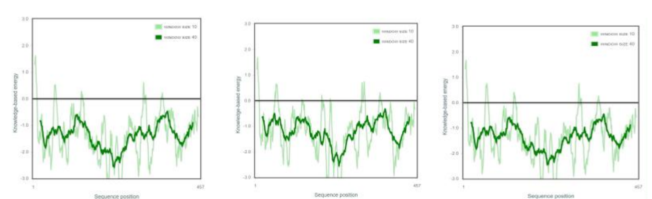
Using AlphaFold, I-TASSER, and SWISS-MODEL, the tertiary structure of the chosen protein was modeled. The quality and dependability of the three-dimensional conformation were confirmed by further validating the projected models with UCLA-SAVES and ProSA-web.



The chosen protein's anticipated 3D structure by AlphaFold, I-TASSER, and SWISS-MODEL



Ramachandran Plot of the selected protein as predicted by AlphaFold, I-TASSER, and SWISS-MODEL



The examination of local model quality for the AlphaFold, I-TASSER, and SWISS-MODEL.

Table 2. Plot statistics

Characteristics	Value		
	AlphaFold	I-TASSER	SWISS-MODEL
Residues in most favored regions	366 (92.4%)	296 (74.7%)	371 (93.7%)
Residues in additional allowed regions	28 (7.1%)	71 (17.9%)	24 (6.1%)
Residues in generously allowed regions	2 (0.5%)	19 (4.8%)	1 (0.3%)
Residues in disallowed regions	0	10 (2.5%)	0
Number of glycine residues	33	33	33

Validation of Tertiary Structure (Ramachandran Plot Analysis)

The quality of the predicted protein structures was evaluated using the Ramachandran plot analysis. The highest quality structure was the SWISS-MODEL one, which had 0% of residues in forbidden regions and 93.7% in preferred regions. I-TASSER displayed 2.5% in forbidden regions, while AlphaFold had 92.4% and 74.7% of residues in preferred regions, respectively.

SWISS-MODEL showed very few additional authorized and generously allowed regions (6.1% and 0.3%), but AlphaFold and I-TASSER showed somewhat more.

CONCLUSION

- ➢ One important opportunistic pathogen that is becoming more and more multidrug-resistant is Acinetobacter baumannii.
- ➢ The protein under analysis has more negatively charged residues and is thermostable, hydrophilic, and stable.
- ➢ The siroheme synthase is present. CysG domain, essential for the biosynthesis of siroheme.
- ➢ GO studies and protein-protein interactions reveal a variety of molecular and biological functions.
- ➢ α-helices dominate secondary structure, although SWISS-MODEL produces the most dependable 3D structure.

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