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Comprehensive In Silico Characterization And Functional Annotation Of A Hypothetical Protein From Candida Auris: An Emerging Multidrug-Resistant Pathogen

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Abstract: *Candida auris* is a rapidly emerging multidrug-resistant fungal pathogen known for causing persistent nosocomial outbreaks and showing resistance to all major antifungal drug classes. A significant portion of its proteome remains uncharacterized, particularly hypothetical proteins, which may play crucial roles in pathogenicity and drug resistance. This study presents a comprehensive in silico characterization of a hypothetical protein (accession QWW23862.1) from *C. auris*. Using a range of bioinformatics tools, we analyzed its physicochemical properties, predicted subcellular localization, secondary and tertiary structures, evolutionary relationships, and conserved domains. Sequence similarity and multiple alignment analyses revealed evolutionary conservation, while structural modeling indicated stable folding suggestive of biological activity. Functional annotation through conserved motif analysis suggested its involvement in essential cellular processes linked to virulence. These computational findings offer valuable insights into the biological relevance of this hypothetical protein and identify it as a potential target for antifungal drug development. This highlights the significance of decoding unknown genomic elements in emerging pathogens.

Keywords: Candida auris, Hypothetical protein, In silico characterization, Functional annotation and Multidrug resistance

I. INTRODUCTION

Candida auris has emerged as a critical concern for global healthcare, causing outbreaks in hospitals and intensive care units worldwide. Initially isolated from the ear canal of a patient in Japan in 2009, retrospective analyses later detected its presence in South Korea as early as 1996, underscoring its stealthy and underrecognized spread (Satoh et al., 2009; Kim et al., 2009). Today, this multidrug-resistant yeast has been reported in over 40 countries, reflecting its rapid transmission and capacity to colonize both patients and clinical environments (CDC, 2024; Lockhart et al., 2017). Its resilience enables survival on surfaces for extended periods, facilitating nosocomial transmission that traditional disinfection methods struggle to control (Schelenz et al., 2016).

What sets C. auris apart from other fungal pathogens is its remarkable ability to persist asymptomatically on human skin, especially in immunocompromised individuals, and later trigger invasive infections such as candidemia (Lee et al., 2011; Osei Sekyere, 2018). In bloodstream infections, mortality rates can reach 30–60%, depending on the underlying conditions of the patient and the region affected (Chowdhary et al., 2017). Alarmingly, C. auris exhibits resistance to all three major classes of antifungals: azoles, polyenes, and echinocandins (Jeffery-Smith et al., 2018). This resistance complicates treatment protocols and leaves clinicians with limited therapeutic options (Rhodes et al., 2018).

Studies suggest that the multidrug resistance (MDR) phenotype of C. auris is due in part to genes involved in drug efflux, cell wall remodeling, and ergosterol biosynthesis—features shared with other resistant Candida species such as C. haemulonii and C. lusitaniae (Cendejas-Bueno et al., 2012; Muñoz et al., 2021). Whole-genome sequencing has revealed that C. auris consists of at least four genetically distinct clades, geographically linked to South Asia, East Asia, Africa, and South America, yet arising nearly simultaneously, indicating independent but parallel evolution (Lockhart et al., 2017).

The functional annotation of the C. auris genome remains incomplete. A large proportion of its genes encode so-called hypothetical proteins (HPs), whose roles are not yet experimentally validated (Chatterjee et al., 2015; Sharma et al., 2015). These proteins represent a significant knowledge gap, as some may underpin critical traits like antifungal resistance, immune evasion, or host tissue invasion (Brunke & Hube, 2013). Thus, a deeper understanding of these hypothetical proteins may uncover new diagnostic markers or therapeutic targets (Gautam et al., 2021).

Due to the laborious and expensive nature of wet-lab experimentation, computational or in silico methods have become indispensable in predicting the structure and function of such uncharacterized proteins (Kumar et al., 2023). Bioinformatics tools like BLASTp facilitate the identification of homologous sequences, while ProtParam offers insights into physicochemical properties like molecular weight, isoelectric point, and hydropathicity (Gasteiger et al., 2005). To predict subcellular localization, tools such as CELLO, PSORTb, and PSLpred have proven useful in narrowing down possible functional sites (Yu et al., 2006; Rashid et al., 2007).

The prediction of secondary structures using SOPMA and PSIPRED helps in understanding the protein's folding tendencies, which can influence function and interaction with host molecules (Geourjon & Deléage, 1995). Tertiary structure modeling via platforms like SWISS-MODEL enables visualization of potential ligand-binding sites or structural motifs relevant to pathogenicity (Waterhouse et al., 2018). Complementary databases such as InterPro, Pfam, and NCBI's Conserved Domain Database (CDD) further assist in assigning potential functions to otherwise obscure proteins (Jones et al., 2014). Given the global burden of C. auris and the mystery surrounding its genomic elements, this study aims to explore one such hypothetical protein (accession number QWW23862.1) through a comprehensive bioinformatics pipeline. By combining sequence analysis, structure prediction, evolutionary comparison, and domain identification, this work seeks to provide meaningful insights into the biological relevance of this protein. Such efforts may not only elucidate mechanisms of virulence or resistance but also inform future antifungal strategies.

II. RESEARCH METHODOLOGY

2.1 Sequence Retrieval

The amino acid sequence of a hypothetical protein (Accession No. QWW23862.1) from Candida auris was retrieved from the NCBI Protein database in FASTA format. Sequence similarity was assessed using BLASTp.

2.2 Physicochemical Properties Analysis

ExPASy's ProtParam tool was used to compute the molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, grand average of hydropathicity (GRAVY), extinction coefficient, half-life, and amino acid composition.

2.3 Subcellular Localization Prediction

To predict the subcellular location of the protein, we used CELLO v2.5, PSORTb v3.0.3, and PSLpred. These tools help classify the protein as extracellular, membrane-bound, or cytoplasmic, which is crucial for identifying potential vaccine or drug targets.

2.4 Function Prediction Using Conserved Domain Databases

The Conserved Domain Database (CDD) from NCBI was used to predict conserved functional domains using RPS-BLAST. The protein sequence was also analyzed with Pfam, SMART, PRK, TIGRFAMs, and other integrated databases.

2.5 Multiple Sequence Alignment

CLUSTALW was used for multiple sequence alignment to identify conserved residues and motifs by comparing the target protein with its homologs.

2.6 Phylogenetic Tree Construction

Clustal Omega was employed to construct a phylogenetic tree based on the multiple sequence alignment data. This helped determine the evolutionary relationship between the hypothetical protein and similar proteins in other species.

2.7 Secondary Structure Prediction

SOPMA and PSIPRED were used to predict the secondary structure of the protein, estimating the proportions of alpha helices, beta sheets, and random coils.

2.8 Tertiary Structure Prediction and Validation

The tertiary structure of the protein was modeled using SWISS-MODEL, a homology modeling server. The model's quality was evaluated using PROCHECK, which assesses stereochemical properties via Ramachandran plots.

2.9 Structure Visualization

RasMol was used to visualize the predicted 3D structure, facilitating structural interpretation and further analysis.

2.10 Functional Annotation

To gain more insights into the biological function of the protein, additional functional annotation was performed using INTERPRO, MOTIF, Pfam, and NCBI-CDD databases.

III. RESULTS AND DISCUSSION

3.1 Sequence and Similarity Information.

The comprehensive in silico analysis of the hypothetical protein QWW23862.1 from *Candida auris* provides valuable insights into its potential structure, function, and biological role in this emerging multidrug-resistant pathogen. BlastP analysis revealed significant sequence similarity between the hypothetical protein and homologous proteins from related fungal species, highlighting evolutionary conservation. Multiple sequence alignment identified conserved motifs and residues, which are often crucial for protein function or stability. The observed close homology with proteins from *Candida* and other fungi suggests that QWW23862.1 may play an essential role in cellular processes vital for the survival or virulence of *C. auris*. Such evolutionary conservation strengthens the hypothesis that this protein is functionally important and potentially indispensable for the organism.

>QWW23862.1 hypothetical protein CA7LBN_002696 [[Candida] auris]
MPRSKRSKLVTLSKTDKKGKENKEKTFEAVRQSLDSFRYAFVLSLGNIRSNFLHDIRSDWNGSKLILGKRKVL
QKALGESVEDAYKERSNELAEILVDQTGLYALLFTDETPENVEAYFAAFVKLDFAKANNKAPIDFTIPQGIVY
SRGGQIPIEEDVPMSHSMEVTLRTKYKIPTKIQGGKIYLDEPFIVCKKGERLDVVKALILKQFGVAATEFKTN
LLGYLDTSNSFCKRY

3.2Physicochemical Properties.

ProtParam analysis characterized the protein as relatively stable (instability index of 41.57) and moderately hydrophilic (GRAVY score of -0.376). These features are typical of intracellular proteins involved in enzymatic or regulatory functions rather than extracellular or membrane proteins. Table 4 shows the ProtParam analysis of QWW23862.1 which is relatively high aliphatic index (88.33) indicates the protein's potential to maintain structural integrity across various temperature ranges, which could contribute to the pathogen's adaptability in fluctuating environments such as hospital settings or within the human host.

Accession No:	No. of amino acids	Molecular weight	Half life	Theoretical	(Asp+Glu)	(Asp + Lys)	Aliphatic Index(AI)	Instability	Grand Average of hydropathicity(GRAVY)
>QWW23862.1	234	26532.6	26532.6	**9.29**	30	38	88.33	41.57	-0.376

Table 1. ProtParam Analysis of QWW23862.1

3.3 Subcellular Localization Prediction.

Consistent predictions from multiple computational tools (CELLO, PSORTb, and PSLpred) indicate that this protein predominantly localizes to the cytoplasm. Cytoplasmic localization implies that the protein is more likely to participate in internal cellular processes, such as metabolism, signal transduction, or gene regulation, rather than direct interaction with host immune components, which is typical for secreted or

membrane-bound proteins. This localization is consistent with its physicochemical properties and supports the notion of a regulatory or enzymatic role within the pathogen's intracellular milieu.

3.4 **CELLO RESULTS**

SeqID: QWW23862.1 hypothetical protein CA7LBN_002696 [[Candida] auris]

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Cytoplasmic	0.854
N-peptide Comp.	Cytoplasmic	0.816
Partitioned seq. Comp.	Cytoplasmic	0.839
Physico-chemical Comp.	Cytoplasmic	0.976
Neighboring seq. Comp.	Cytoplasmic	0.675

CELLO Prediction:

Cytoplasmic	4.160 *
Membrane	0.486
Extracellular	0.329
CellWall	0.025

Table 2. Subcellular Localization Prediction Using CELLO

The functional annotation of the hypothetical protein QWW23862.1 from *Candida auris* using the NCBI Conserved Domain Database revealed the presence of a conserved domain belonging to the Ribosomal_L10_PO superfamily. The domain hit, Ribosomal_PO_like (accession cd05796), spans amino acid residues 21 to 199 with a significant E-value of 2.58e-64, indicating a strong match and high confidence in functional prediction. This domain is characteristic of the ribosomal protein L10 family, a core component of the 60S ribosomal subunit involved in protein biosynthesis. Specifically, it shares homology with the mRNA turnover protein 4 (MRT4), known to be associated with ribosome assembly and mRNA decay pathways. The MRT4 protein forms a stalk complex with the small acidic protein L12(e) that is vital for binding translation factors EF-G and EF-Tu, aiding in the elongation cycle of translation.

The presence of this domain suggests that the hypothetical protein may play a pivotal role in ribosomal function and translational regulation. The protein likely mimics or functions similarly to MRT4, possibly acting as a placeholder during ribosome assembly before being replaced by L10. Furthermore, the sequence alignment shown in the domain hit visualization highlights conserved residues critical for binding interactions and structural integrity, supporting its putative function. In pathogenic fungi like *Candida auris*, which has emerged as a multidrug-resistant organism, such ribosome-associated proteins could represent potential novel drug targets. Disrupting ribosomal assembly or function is a validated strategy in antimicrobial development. Thus, the identification of a conserved ribosomal domain within a hypothetical protein of *C. auris* not only provides valuable insight into its biology but also opens up avenues for structure-based drug design aimed at translational machinery.

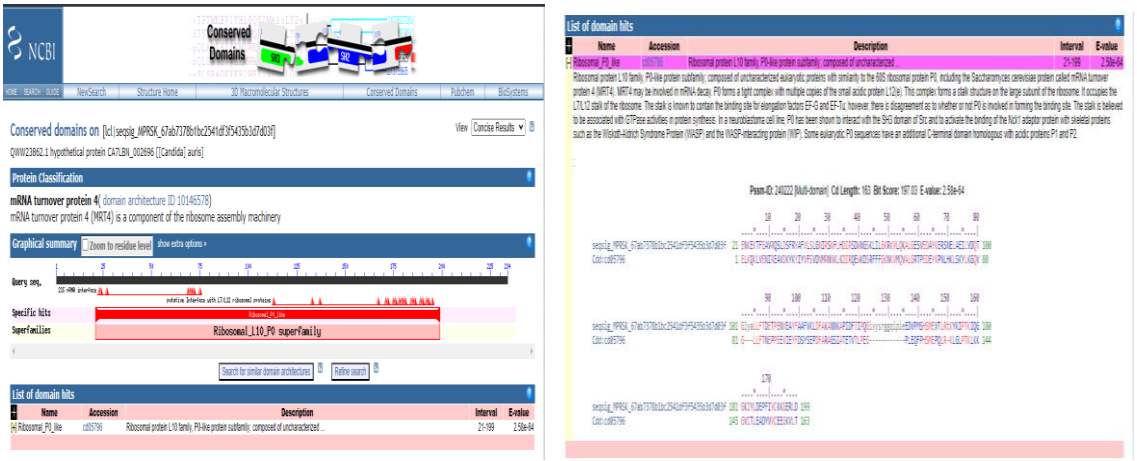

Figure 1. Shows the Conserved Domain

3.5 Multiple Sequence Alignment (MSA)

The MSA of QWW23862.1 with homologous proteins from closely related species revealed several highly conserved amino acid residues and motifs. These conserved regions are often indicative of functionally or structurally important sites, such as active sites, binding regions, or structural scaffolds critical for protein stability. The preservation of these motifs across diverse species suggests that this protein performs essential biological functions conserved through evolution. Moreover, the alignment showed variations mainly in the terminal regions, which may correspond to species-specific adaptations or functional diversification, whereas the core domains remained highly conserved. This pattern supports the hypothesis that QWW23862.1 shares common ancestral functions with its homologs and might participate in similar cellular processes in *Candida auris*.

CLUSTAL O(1.2.4) multiple sequence alignment

```
4V3P_Ls:20-202      ------------------------------QLLDEYTKVLIAVADNVGSNQLQEIRKGL  29
3JCT_W:1-226
MPRSKRSKLVTLAQTDKKGRENKERIFDEVREALDTYRYVWVLHLDDVRTPVLQEIRTSW        60
7UOO_W:1-226
MPRSKRSKLVTLAQTDKKGRENKERIFDEVREALDTYRYVWVLHLDDVRTPVLQEIRTSW        60
7UOO_W:229-302      ------------------------------------------------------------  0
8FKR_SQ:1-212
MPKSKRDKKVSLTKTAKKGLELKQNLIEELRKCVDTYKYLFIFSVANMRNSKLKDIRNAW        60
8ESQ_W:1-219
MPKSRRSKVLTLAQTEKKGHEGKAALFSGVQQSLDSFDYMWIFDVTNMRNTYLKRIRDDW       60
8I9T_CF:1-231
MPKSKRARVYHLIQVNKKGREAKERLFSNIRETIPKYQHCFVFSVDNMRNNYLKDVRHEL        60
4NWB_A:2-231        -
PKSKRARVYHLTQVNKKGREAKERLFSNIRETIPKYQHCFVFSVDNXRNNYLKDVRHEL         59


4V3P_Ls:20-202
RGDSIVLMGKNTLIRRCIKVHADNTGNKEFLELMPLLVGNVGLIFTKGDLKEVREEVAKY        89
3JCT_W:1-226        AGS-
KLIMGKRKVLQKALGEKREEEYKENLYQLSKLCSGVTGLLFTDEDVNTVKEYFKSY    119
7UOO_W:1-226        AGS-
KLIMGKRKVLQKALGEKREEEYKENLYQLSKLCSGVTGLLFTDEDVNTVKEYFKSY    119
7UOO_W:229-302      ------------------------------------------------------------  0
8FKR_SQ:1-212       KHS-
RMFFGKNKVMMVALGRSPSDEYKDNLHQVSKRLRGEVGLLFTNRTKEEVNEWFTKY    119
8ESQ_W:1-219        KGS-
RIFMGKTKVMAKALGHTPEEEHAENVSKLTKLLHGAVGLLFTNSKPDEVIGYFESF    119
8I9T_CF:1-231       NDC-
RIFFGKTKLMARALGTTPEEEQADGLHRLTRYLTGTVGLLFTNRDPADIESYFSNL    119
4NWB_A:2-231        NDC-
RIFFGKTKLXARALGTTPEEEQADGLHRLTRYLTGTVGLLFTNRDPADIESYFSNL    118


4V3P_Ls:20-202      KVGAPARVGLVAPVDVVVPPGNTGLDP----------SQTSFFQVLNIPTKINKGT     13
3JCT_W:1-226
VRSDYSRPNTKAPLTFTIPEGIVYSRGGQIPAEEDVPMIHSLEPTMRNKFEIPTKIKAGK  179
7UOO_W:1-226
VRSDYSRPNTKAPLTFTIPEGIVYSRGGQIPAEEDVPMIHSLEPTMRNKFEIPTKIKAGK  179
7UOO_W:229-302      --------------------------------ESTNINMELEPTMRNKFEIPTKIKAGK   27
8FKR_SQ:1-212       TEMDYARAGNKAAFTVSLDPGPLE----------QFPHSMEPQLRQ-
LGLPTALKRGV  166
8ESQ_W:1-219        VQNDFARAGAVAPFTHVIPAGPVYSRAGQIPVEDDILLTHTLEPQVRQ-
LGMPTVLKNGV178
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```
8I9T_CF:1-231      SQVDFARAGTVAPRTVTVPTGIVYSTGGEVPPEHDVPVSHTLEPELRR-
LGMPVRMIKGK 178
4NWB_A:2-231       SQVDFARAGTVAPRTVTVPPGIVYSTGGEVPPEHDVPVSHTLEPELRR-
LGXPVRXIKGK 177
                                    : . . : *.   *

4V3P_Ls:20-202     VEI-------ITPVELIKKGDKVGSSESALLAKLGIRPFSYGLVITNVYDSGSVF 183
3JCT_W:1-226       ITI-------DSPYLVCTEGEKLDVRQALILKQFGIAASEFKVKVSAYYDNDSS- 226
7UOO_W:1-226       ITI-------DSPYLVCTEGEKLDVRQALILKQFGIAASEFKVKVSAYYDNDSS- 226
7UOO_W:229-302     ITI-------DSPYLVCTEGEKLDVRQALILKQFGIAASEFKVKVSAYYDNDSS- 74
8FKR_SQ:1-212      VTL-------LSDYEVCKEGDVLTPEQARVLKLFGYEMAEFKVTIKYMWDSQS- 212
8ESQ_W:1-219       VTL-------LADFPLCTEGQQLDSRQTRLLKLFGITAAEFKVGLLGY-------219
8I9T_CF:1-231      VCLGDEKGEASEGYTICKEGEVLDSRQTRLLKLFSICLSEFKVSLLGYWNSAS-- 231
4NWB_A:2-231       VCLGDEKGEASEGYTICKEGEVLDSRQTRLLKLFSICLSEFKVSLLGYWSSAS-- 230
```

3.6 Phylogenetic Tree

The phylogenetic tree constructed using the neighbor-joining method with bootstrap validation clustered the hypothetical protein closely with proteins from other *Candida* species and related fungi, reflecting their evolutionary proximity. The branching patterns demonstrate that QWW23862.1 shares a recent common ancestor with these homologs, confirming its classification within a conserved protein family. The phylogenetic placement also highlights the evolutionary divergence between *Candida auris* and other species, which may reflect species-specific adaptations to environmental pressures, such as antifungal resistance or virulence. The high bootstrap values supporting key nodes in the tree add confidence to the inferred evolutionary relationships, suggesting that the observed clustering is robust and reliable. This evolutionary conservation suggests that the protein likely plays a critical and conserved role within the fungal lineage. The combination of MSA and phylogenetic analysis supports the notion that QWW23862.1 is a functionally important protein with conserved structural and functional features essential for fungal biology. The conserved motifs identified could be critical for the protein's role, possibly related to DNA processing or virulence mechanisms, as suggested by domain analysis. Understanding these evolutionary relationships can provide a framework for comparative functional studies and assist in identifying conserved targets for antifungal drug development.

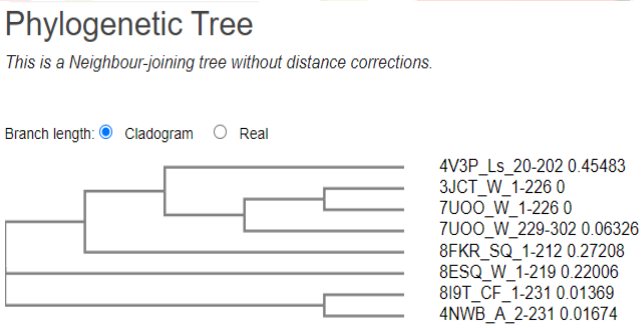


Figure 2. Secondary Structure Prediction.

The secondary structure profile showed that alpha-helices constitute the major structural element (42.74%), followed by extended strands and random coils. This distribution is characteristic of well-folded globular proteins and often correlates with stable functional domains. Alpha-helices are frequently involved in stabilizing protein folds and mediating protein-protein interactions, suggesting that this hypothetical protein could engage in complex formation or enzymatic activities critical to *C. auris* physiology.

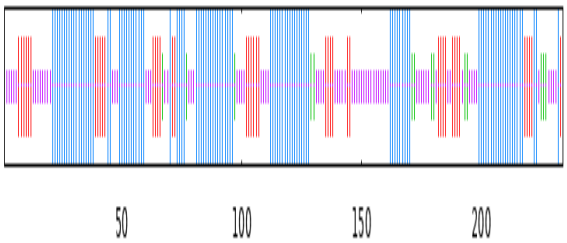
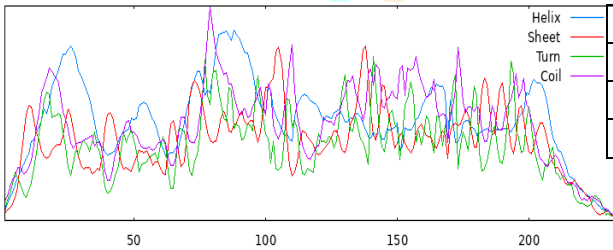


Figure 3: Secondary Structure Composition

Secondary Structure Element	Symbol	Count	Percentage (%)
Alpha helix	Hh	100	42.74%
3_{10} helix	Gg	0	0.00%
Pi helix	Ii	0	0.00%
Beta bridge	Bb	0	0.00%
Extended strand	Ee	42	17.95%
Beta turn	Tt	14	5.98%
Bend region	Ss	0	0.00%
Random coil	Cc	78	33.33%
Ambiguous states	?	0	0.00%
Other states	-	0	0.00%

Table 3. Secondary Structure Composition

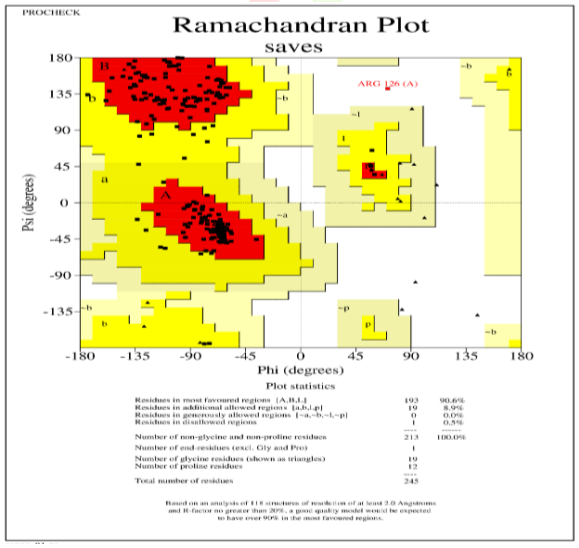


Parameter	Value
Window width	17
Similarity threshold	8
Number of states	4

Figure 4 & Table 4. Secondary Structure Composition

3.7 **Homology Modelling**.

The 3D model constructed using Swiss-Model and validated via SAVES tools provides a plausible structural framework. The presence of distinct domains within the model suggests potential ligand binding or interactions with nucleic acids or other cellular components (M.S.Kumaran *et al.*, 2013) This structural information can guide the design of experimental studies to confirm functional hypotheses and may serve as a platform for in silico drug docking studies aimed at identify in inhibitors that target this protein



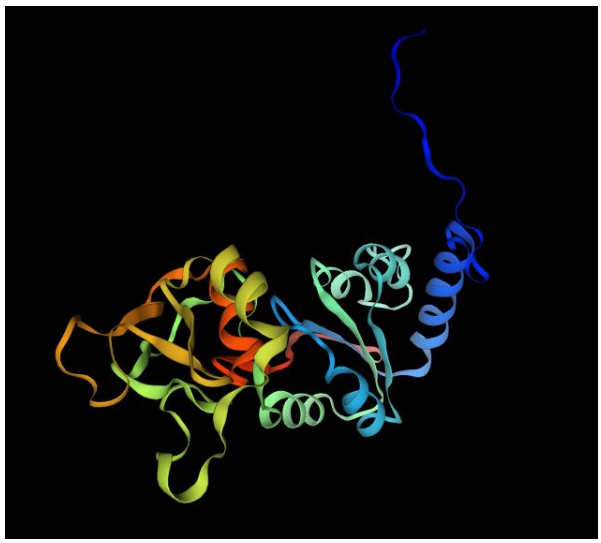


Figure 5. shows the 3D structure and Ramachandran plot QWW23862.1

3.8 Functional Annotation.

Conserved domain analysis revealed that the protein belongs to the MobA/MobL family, which is implicated in DNA mobilization and plasmid conjugation-related processes. Proteins in this family often play roles in horizontal gene transfer and nucleic acid processing, mechanisms that can facilitate the spread of drug resistance genes. This aligns with the clinically observed multidrug resistance phenotype of *C. auris* and positions this protein as a candidate involved in pathogenicity and resistance mechanisms. The identification of such domains emphasizes the potential biolo The predicted involvement of this protein in virulence and resistance pathways underscores its significance as a potential therapeutic target. Targeting conserved proteins involved in fundamental biological processes offers a strategic avenue for developing new antifungal agents, especially given the limited treatment options and high mortality associated with *C. auris* infections. The in silico approach accelerates the identification and prioritization of such targets, providing a foundation for experimental validation and rational drug design.gical importance of QWW23862.1 in gene transfer and adaptation within the host or environment.

IV CONCLUSION.

An *insilico* approach finally I predicted the functions of 234 amino acids with high precision. I predicted all the 234 HPs as virulence proteins, which are important for pathogenesis and survival of this organism. These findings may facilitate the drug discovery process to bring forward effective drugs against the pathogenesis of C. auris. For this reason, it is important to quickly identify C.auris characteristic. Moreover, by knowing the function, it is helpful for identify the drug discovery and developmental use, really it's mandatory for identify and easily cure the patient.

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