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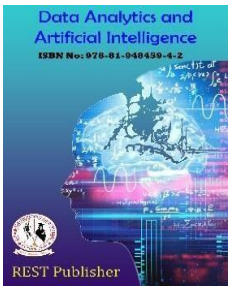

Comparative Genomics of Translation Initiation: Ortholog Mapping from Candida Albicans to Candida Auris and Saccharomyces Cerevisiae

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Abstract*: Translation initiation is a critical and evolutionarily conserved step in eukaryotic gene expression, but its functional conservation across fungal pathogens remains poorly characterized. In this study, we explore the conservation of translation initiation mechanisms across three fungal species: the well- annotated Candida albicans (SC5314), two genetically distinct clinical strains of Candida auris (B8441 and B11221), and the model yeast Saccharomyces cerevisiae (S288C). Using a computational bioinformatics pipeline, we integrate or- thogroup clustering (via OrthoFinder [1]), Gene Ontology (GO)- based annotation, domain prediction, and reciprocal BLAST hit analysis to identify and characterize orthologs involved in translation initiation. The analysis is guided by key GO terms including GO:0006413 (translational initiation), GO:0003743 (initiation factor activity), and others associated with translation complexes. We construct species-wise orthogroup presence matrices and visualize patterns of conservation using heat maps, UpSet plots, and force-directed network diagrams. Our results reveal that several essential translation initiation components—such as eIF1, eIF4E, and DOM34—are strongly conserved across all three species. In contrast, certain factors exhibit lineage- or strain-specific divergence, including variations in domain architecture and gene duplication events. Interestingly, although the two C. auris strains exhibit high genome-wide sim ilarity, orthogroup conservation specific to translation initiation genes shows more selective retention. This study demonstrates the effectiveness of annotation transfer in characterizing functional modules across fungal genomes, highlighting the translational machinery as a conserved and computationally tractable biological system. The pipeline developed here illustrates how genome-scale annotation, homology inference, and functional filtering can be integrated to sup- port hypothesis generation in pathogen genomics. Our findings provide a foundation for future experimental work, including the development of computational pipelines related to ribosome profiling, to validate the regulatory and functional relevance of conserved initiation factors across fungal pathogens.*

Keywords*: Gene Ontology (GO), Functional annotation transfer, Advanced Applications of AI*

1. INTRODUCTION

Translation initiation is a critical and evolutionarily con- served step in eukaryotic gene expression that regulates the efficiency with which proteins are synthesized in response to cellular and environmental signals [2], [3]. This process is or- chestrated by a network of eukaryotic initiation factors (eIFs) and associated regulatory elements that coordinate ribosome recruitment to mRNA. Although the key components of trans- lation initiation have been extensively studied in the model yeast *Saccharomyces cerevisiae* (S288C), their conservation across clinically relevant fungal pathogens, particularly the emergent multidrug-resistant species *Candida auris*, remains incompletely understood [4]. *C. albicans* (SC5314) serves as a well-characterized model for fungal gene regulation and patho- genesis. In contrast, *C. auris* represents a rapidly evolving global health threat due to its resistance to major antifungals and frequent outbreaks in healthcare settings [5]. The species comprises multiple genetically distinct clades; among them, strains B8441 and B11221 are known to differ in key genomic features [6]. Understanding how essential biological processes like translation initiation are conserved or diverge between such strains could offer mechanistic insight into strain-specific phenotypes and inform antifungal drug target discovery.

To address this, we investigate the conservation of transla- tion initiation across three species: *C. albicans*, two strains of *C. auris* (B8441 and B11221), and *S. cerevisiae*. Us- ing orthologous gene groups from the Candida Gene Order Browser (CGOB) [7] and reciprocal best-hit (RBH) mappings from the Candida Genome Database (CGD) [8], we focus on genes annotated with Gene Ontology (GO) terms related to translation initiation (e.g., GO:0006413, GO:0003743). Our analysis leverages presence/absence matrices, domain conser- vation, and visualization through heatmap plots to contrast genome-wide versus pathway-specific conservation between the two *C. auris* strains.

This framework not only highlights conserved components of the translation initiation machinery, but also reveals subtle divergences that may influence strain-specific adaptation or drug susceptibility. Through comparative functional annotation transfer, we aim to improve the understanding of core molec- ular processes in emerging fungal pathogens. The broader goal of this work is twofold: first, to validate orthology-based annotation transfer as a practical strategy for emerging fungal pathogens, and second, to explore whether conservation of essential processes like translation initiation can inform future experimental and therapeutic studies.

TABLE 1. Summary of Key Orthogroups and Associated GO Terms for Translation Initiation

Ortho group ID	GO Term	GO Description	Species Present	# Genes
OG0006413	GO:0006413	Translational initiation	*C. auris*, *C. albicans*, *S. cerevisiae*	4
OG0003743	GO:0003743	Translation initiation factor activity	*C. auris*, *C. albicans*, *S. cerevisiae*	4
OG0001731	GO:0001731	Formation of translation preinitiation complex	*C. auris*, *C. albicans*	3
OG0005852	GO:0005852	Eukaryotic translation initiation factor 3 complex	*C. auris*, *S. cerevisiae*	3
OG0015934	GO:0015934	Large ribosomal subunit	*C. auris*, *C. albicans*, *S. cerevisiae*	5
OG0016281	GO:0016281	Translation initiation factor binding	*C. albicans*, *S. cerevisiae*	3
OG0031369	GO:0031369	Protein complex localization to nucleus	*C. auris*, *C. albicans*, *S. cerevisiae*	4
OG0006412	GO:0006412	Translation	*C. auris*, *C. albicans*, *S. cerevisiae*	6

2. METHODOLOGY

Orthologous gene group data were obtained from the Can- dida Gene Order Browser (CGOB) [7], while reciprocal best- hit (RBH) gene pairs were downloaded from the Candida Genome Database (CGD) [8] for three key organisms: *Can- dida albicans* SC5314, *Candida auris* strains B8441 and B11221, and *Saccharomyces cerevisiae* S288C. GO anno- tations were retrieved from CGD for functional filtering, with a focus on translation initiation-related terms such as GO:0006413 (translational initiation) and GO:0003743 (trans- lation initiation factor activity). All input data were parsed and processed in R (v4.3.1) using packages from the tidyverse ecosystem [9]. Initial data cleaning ensured proper formatting, removal of metadata rows, and alignment of ortholog columns. Gene presence/absence was converted into binary matrices for downstream visualiza- tion.

A. Orthogroup Identification and Filtering

Orthogroups containing genes from all three species were selected using a custom filtering pipeline. Genes with complete orthology mappings across *C. albicans*, both *C. auris* strains, and *S. cerevisiae* were retained for comparative analysis. Ad- ditional filtering focused on orthogroups linked to translation initiation via GO term annotations. Known initiation factors (e.g., eIF1, eIF2, eIF3 subunits) were manually curated and tracked across species.

B. Comparative Genomic Analysis Using UpSet Plots

To examine conservation patterns at multiple scales, we implemented two UpSet plot [10] analyses using the UpSetR package [11]. First, a genome-wide UpSet plot [10] was gener- ated to assess the overall intersection of orthogroups between *C. auris* B8441 and B11221. Second, a translation initiation- specific UpSet plot [10] was created using a curated list of eight GO-annotated orthogroups involved in translational control. By contrasting these two plots, we were able to distinguish genome-wide conservation from the more selective retention of translation-specific genes. Orthogroups found only in one strain were flagged for further functional annotation or domain analysis using InterProScan [12].

C. Visualization and Domain Analysis

Orthogroup overlap patterns were visualized using bar plots, UpSet diagrams, and network graphs. To assess functional conservation, protein domains were annotated using InterPro [13] and compared across species and strains. Visualization outputs were generated with ggplot2, pheatmap, and UpSetR to support interpretation of both conserved and divergent features.

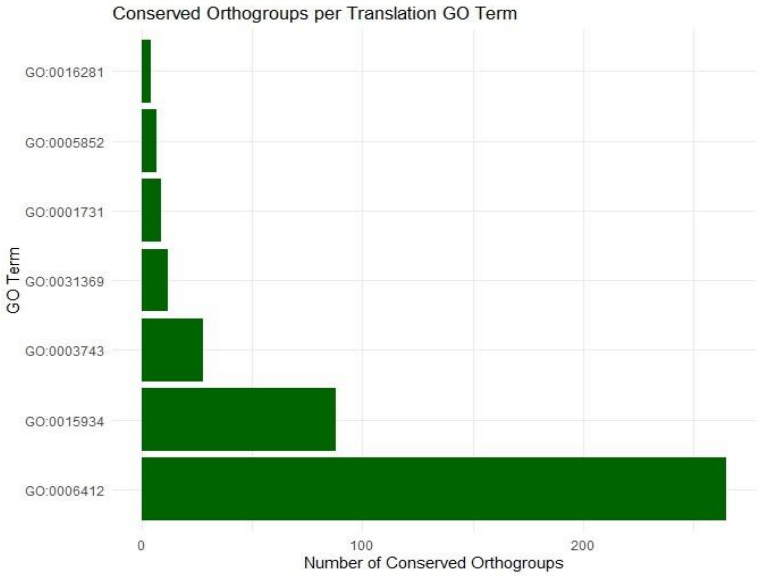

FIGURE 1. Bar plot showing the number of conserved orthogroups identified for each Gene Ontology (GO) term related to translation processes across C. auris,

C. albicans, and S. cerevisiae. GO:0006412 (translation) shows the highest conservation, indicating strong evolutionary preservation of core translational machinery..

D. Comparative Analysis and Annotation

Putative translation initiation orthologs were transferred and analyzed in C. auris. These were mapped to their annotated C. albicans counterparts. Where applicable, functional domains were predicted using InterPro annotations to validate conservation of activity. Differences in gene presence, copy number, and domain structure across C. auris strains and S. cerevisiae were noted.

E. Tools and Environment

All analyses were conducted using R (v4.3+) and RStudio. Packages used included tidyverse, readr, dplyr, and stringr Visualization of gene overlap and data quality was performed with ggplot2 and UpSetR.

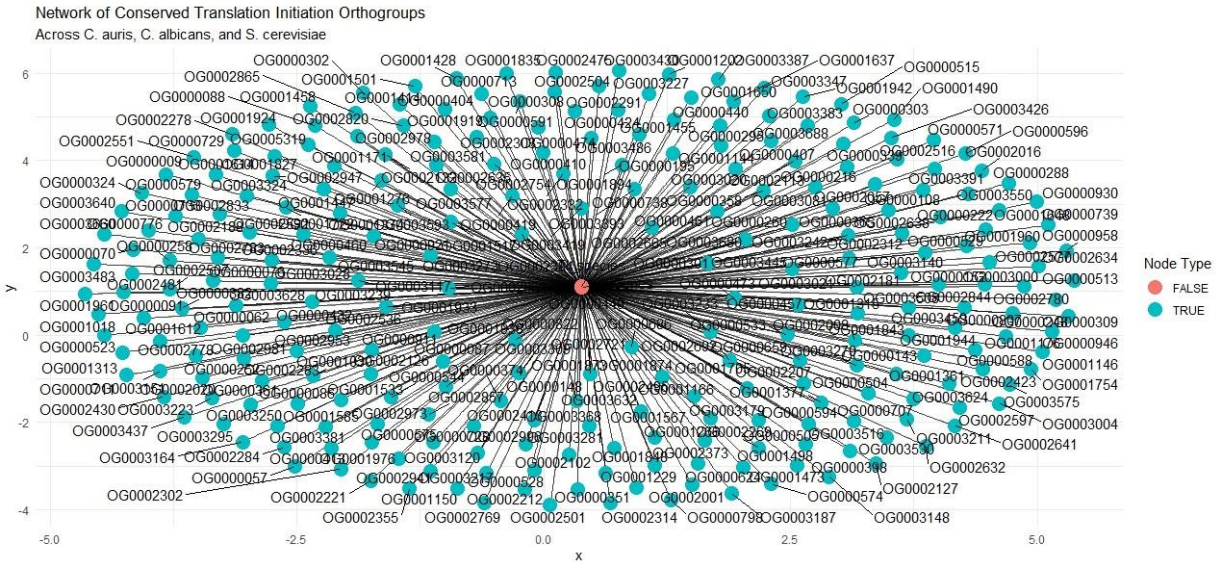

FIGURE 2. Network of conserved translation initiation orthogroups across *C. auris*, *C. albicans*, and *S. cerevisiae*.

3. RESULTS

A. *Identification of Conserved Translation Initiation Genes*

Orthology analysis using OrthoFinder [1] identified mul- tiple orthogroups shared across *Candida albicans* (SC5314), *Candida auris* (B11221 and B8441), and *Saccharomyces cerevisiae* (S288C). As shown in Figure 1 above, we fo- cused on orthogroups associated with Gene Ontology (GO) terms related to translation initiation, including GO:0006413 (translational initiation), GO:0003743 (translation initiation factor activity), and several related functional categories. These correspond to Orthogroups and GO terms listed in Table 1.

B. *Conservation of Key Orthogroups Across Species*

Eight orthogroups were found to be conserved among at least two of the three species, with several present across all three (Table I). Notably, orthogroups OG0006413 and OG0003743 were shared among all species and correspond to canonical translation initiation processes and factors. These findings indicate that core components of the translation initi- ation machinery are conserved across divergent yeast species.

C. *Network Visualization of Orthogroup Conservation*

As shown in Figure 2, to illustrate conservation patterns, we constructed a network graph showing relationships between species and conserved orthogroups. Nodes represent either species-specific gene identifiers or orthogroups, while edges indicate membership in a given orthogroup. The graph high- lights dense interconnectivity between conserved orthogroups and all three species, especially for GO terms directly associated with translation initiation (e.g., GO:0006413, GO:0006412). Orthogroups OG0015934 (large ribosomal subunit) and OG0031369 (protein complex localization to nucleus) also exhibit broad conservation, reinforcing the role of structural and localization factors in conserved initiation mechanisms.

D. *Species-Specific Variation*

Some GO terms were absent in one species, suggesting evolutionary divergence in the peripheral components of the initiation pathway. For example, OG0001731 (formation of the preinitiation complex) was not detected in *S. cerevisiae*, whereas OG0016281 (initiation factor binding) was absent in *C. auris*. These absences may reflect either true biological differences or annotation limitations.

E. *Genome-Wide vs. Translation-Specific Gene Overlap Be- tween C. auris Strains*

To further explore patterns of conservation between Can- dida auris strains B8441 and B11221, we performed a com- parative intersection analysis using UpSet plots (Figure 3). The genome-wide UpSet plot [10] (left) reveals that the vast majority of orthogroups (5,180) are shared between the two strains, indicating broad genomic conservation. A small subset of orthogroups is unique to either B8441 or B11221, potentially reflecting strain-specific gene loss, duplication, or annotation gaps. In contrast, the translation initiation-specific UpSet plot [10] (right) is more focused and shows that only 2 orthogroups related to translation initiation are shared between both strains. This narrower overlap highlights the stringency of conservation at the level of functionally annotated translation initiation genes. Together, these plots reinforce that while overall genomic content is largely conserved between C. auris strains, the subset of genes specifically tied to translation initiation is more restricted, suggesting potential regulatory or structural divergence in this essential pathway. Such differ ences, although subtle, may have functional implications for antifungal susceptibility or stress response in clinical settings.

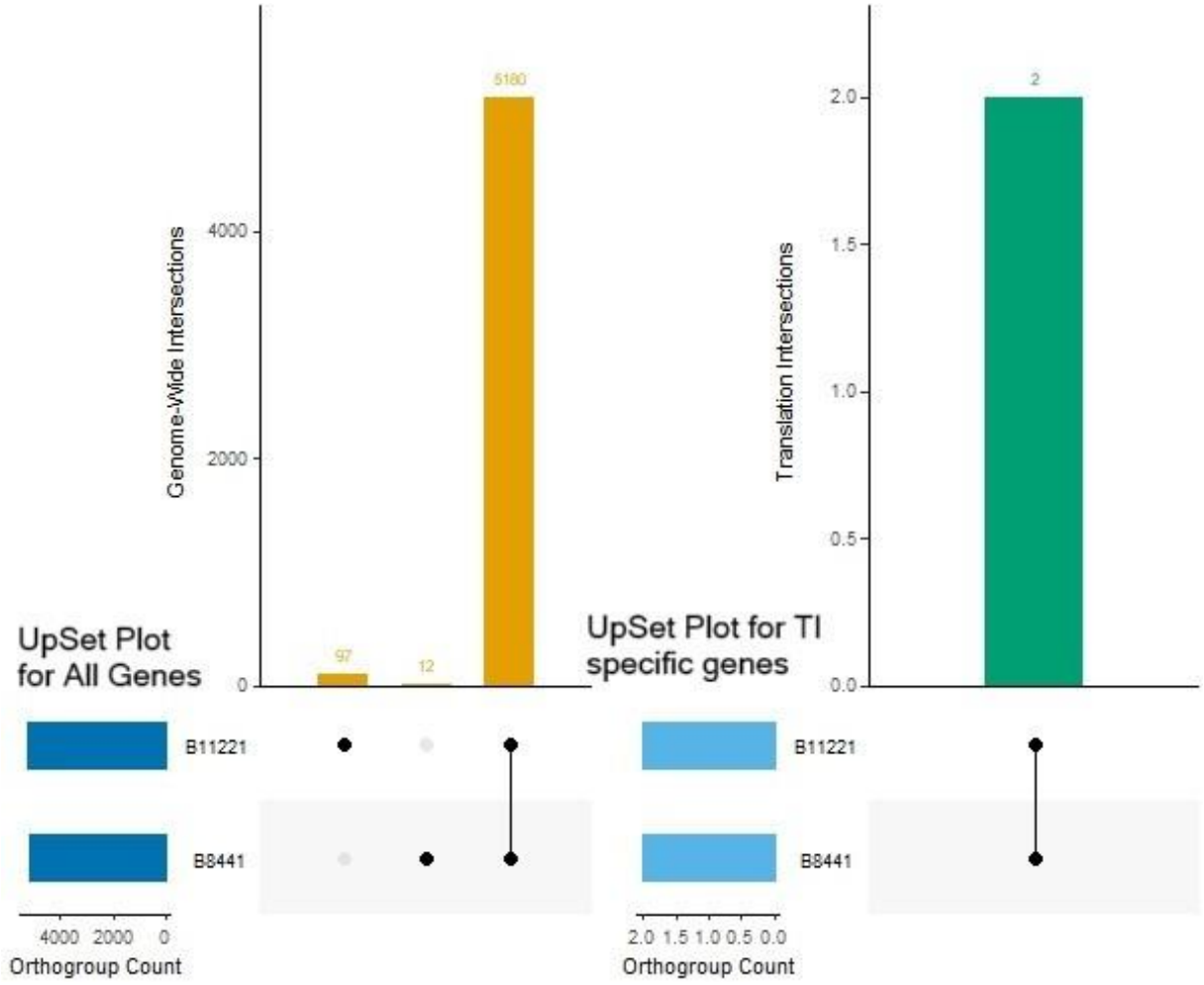

FIGURE 3. Comparative UpSet plots of orthogroup intersections between *Candida auris* strains B8441 and B11221

Figure shoews (Left): Genome-wide UpSet plot [10] showing the distribution of all orthogroups across both strains. A total of 5,180 orthogroups are shared, with a small number unique to each strain, indicating broad genomic conservation. **(Right)**: UpSet plot [10] focused on translation initiation (TI)-specific orthogroups. Only 2 TI-related orthogroups are shared, suggesting that while core translational components are conserved, functional divergence or annotation variability may exist at the pathway-specific level.

4. DISCUSSION

Our analysis of conserved orthogroups associated with translation initiation processes across three fungal species— *Candida auris*, *Candida albicans*, and *Saccharomyces cere- visiae*—highlights key molecular players that are evolutionar- ily maintained, suggesting essential functional roles. Using or- thogroup clustering from OrthoFinder [1] and GO annotations derived from eggNOG-mapper, we identified eight GO terms relevant to translation and initiation machinery. For each GO term, specific genes conserved across the three species were noted, providing insights into the evolutionary conservation of translational control.

For **GO:0006413 (translational initiation)**, orthogroup OG0000001 includes *S. cerevisiae* gene YGR118W (DOM34), which has been shown to play a role in ribosome rescue during translation stress [14]. In *C. albicans*, the ortholog is CR_02740C_A, and in *C. auris B8441*, the gene B9J08_001487 shows strong sequence homology, indicating conserved functionality.

Under **GO:0003743 (translation initiation factor activ- ity)**, orthogroup OG0000015 features TIF1 in *S. cere- visiae*, with homologs CJI97_002731 in *C. albicans* and B9J08_004112 in *C. auris*. These factors are crucial for mRNA cap recognition, and recent studies confirm the essen- tiality of TIF1 in fungal virulence and stress response [15].

GO:0006412 (translation) encompasses a broader set of orthogroups, such as OG0000037, which includes riboso- mal proteins like RPS3 and RPL12. These are universally conserved and central to the ribosome's structural integrity and function. Their presence across all three species reaffirms their role as core components of the translational machinery. Interestingly, under **GO:0016281 (rRNA metabolic process)**, genes like NOP1 in *S. cerevisiae* align with CR_09850W_A in *C. albicans* and B9J08_002972 in *C. auris*, suggesting conservation in ribosome biogenesis pathways. This is con- sistent with prior findings indicating that disruption of rRNA processing can severely attenuate growth and pathogenicity [16].

In addition to functional conservation, the visualization of orthogroup networks (Figure 2) and the UpSet plot [10] of conserved GO terms (Figure 3) reveal strong interspecies overlap, particularly for essential translational components. The network shows tightly connected orthogroups involving translation factors, while the plot highlights that GO:0006413 and GO:0006412 are the most consistently conserved across all species.

These findings support the hypothesis that translational ini- tiation mechanisms are under strong purifying selection, pre- serving both sequence and function. This conservation could be exploited for antifungal targeting, especially as resistance in non-*albicans* Candida species like *C. auris* continues to rise. Future research should investigate whether differential regula- tion of these conserved genes contributes to pathogenicity or environmental adaptation.

To provide evolutionary context to our orthogroup and functional conservation analysis, we constructed a phyloge- netic tree based on concatenated alignments of conserved single-copy orthologs (Figure 4). iTOL was used for tree visualization [17]. As expected, C. auris strains B8441 and B11221 cluster closely, reflecting their recent divergence, while C. albicans and S. cerevisiae appear more distantly related. This topology supports our observation that translation initiation-related genes are largely conserved between the two C. auris strains, yet a small subset of orthogroups shows potential strain-specific variation. The evolutionary distances observed may also correlate with the distribution of strain- specific orthogroups visualized in our UpSet plots (Figure 3).

5. CONCLUSION

This study demonstrates that functional annotation transfer from *Candida albicans* to *Candida auris* can reveal substan- tial conservation in translation initiation mechanisms across fungal species. By leveraging orthogroup clustering from OrthoFinder [1], GO-term annotations, and domain structure validation, we identified eight key orthogroups associated with core translation-related GO terms. Many of these orthogroups, such as those linked to GO:0006413 (translational initiation) and GO:0003743 (initiation factor activity)—were conserved across *C. albicans*, *C. auris* (both B8441 and B11221), and *S. cerevisiae*, supporting the evolutionary conservation of these essential molecular functions.

Network visualization and UpSet analysis highlighted both conserved and divergent components of the translational ma- chinery. While genome-wide orthogroup conservation between *C. auris* strains was extensive, a more selective retention of translation initiation orthologs was observed. Notably, cer- tain orthogroups such as OG0001731 (translation preinitiation complex formation) were absent in *S. cerevisiae*, while others like OG0016281 (initiation factor binding) were missing in *C. auris*, suggesting possible functional divergence or annotation gaps.

These findings support the hypothesis that translation initi- ation is under strong purifying selection [18]–[20], preserving its core components even across phylogenetically distant fungi. The ability to trace conserved molecular components through orthologous relationships enhances our understanding of fun- gal biology and presents opportunities for antifungal drug dis- covery [21], [22]. Specifically, conserved translation initiation factors (e.g., eIF1, eIF4E, DOM34) may serve as targets for therapeutic intervention, particularly given the emergence of multidrug-resistant *C. auris* strains [23].

A. Future Work

Future investigations should focus on both computational refinement and experimental validation of translation initiation mechanisms in *Candida auris* and related species:

- **Expanded orthology inference:** Include more fungal genomes and clade-specific strains in OrthoFinder [1] analysis to better define conserved versus species-specific initiation factors.
- **Domain-level comparison:** Use tools such as Inter- ProScan [12] or Pfam to compare domain architectures of conserved initiation factors across species.
- **RNA structure-aware annotation:** Integrate mRNA sec- ondary structure prediction with GO annotation to iden- tify initiation factors sensitive to structured 5' UTRs, as implicated by DEAD-box helicases Ded1 and Dbp1 [24].

- **Expression-driven prioritization:** Utilize RNA-seq and ribosome profiling data to rank orthogroups by transcriptional activity or translational efficiency under stress or drug exposure [25], [26].
- **Experimental validation:** Use gene knockout or CRISPRi screening in *C. auris* to test the essentiality and functional roles of predicted initiation factors [27].

These strategies will help validate conserved regulatory el- ements and uncover fungal-specific adaptations in translation, informing future antifungal target discovery. Future efforts should expand upon this study by incor- porating additional fungal species and deeper experimental validation. Ribosome profiling and transcriptome-wide trans- lation efficiency assays could elucidate whether the observed gene conservation translates into conserved regulatory function under stress or antifungal treatment. In addition, integrating expression datasets from pathogenic versus non-pathogenic conditions could uncover context-specific regulation of con- served eIFs. The approach can also be extended to explore divergence within translation elongation and termination, or other con- served pathways such as ribosome biogenesis. Finally, leverag- ing structural bioinformatics tools to predict functional disrup- tion in strain-specific paralogs may yield insights into adaptive evolution and antifungal resistance mechanisms. These direc- tions will deepen our understanding of fungal translational control and facilitate the development of targeted antifungal strategies.

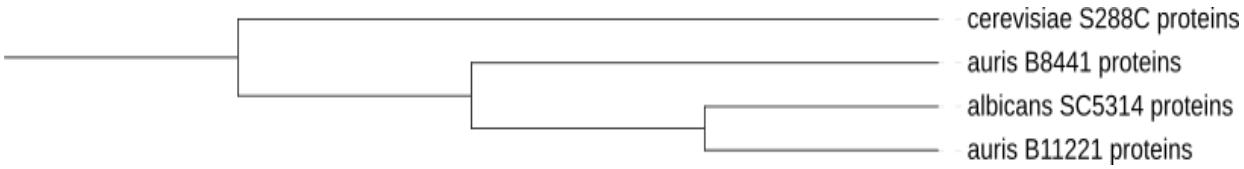

FIGURE 4. Species phylogeny of four fungal genomes based on single-copy orthologs.

The maximum likelihood tree was generated using OrthoFinder [1] from concatenated alignments of conserved single-copy orthologous proteins. *S. cerevisiae* appears as the outgroup, followed by *C. albicans*, while *C. auris* strains B8441 and B11221 form a tight clade, indicating their close evolutionary relationship. Branch lengths are proportional to inferred amino acid substitutions.

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