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*Full length Paper*

# Comparative Analysis of Cytochrome P450 Genes in *Cryptococcus Terricola* JCM 24523 v1.0 *and Cryptococcus Curvatus* ATCC 20509 v1.0

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**ABSTRACT**

Cryptococc*us terricola* and *Cryptococcus curvatus ATCC 20509 v1.0* are closely related fungal species that differ in their metabolic capabilities and adaptation to their respective habitats. *C.terricola* can utilize starch (polysaccharides) to produce biolipids, whereas *C. curvatus* can utilize a mixture of xylose, glucose, and L-arabinose to produce lipids. One of the challenges of the agricultural and food processing industries is dealing with waste biomass, which constitutes a challenge to the environment. Cytochrome P450 (CYP) is a large family of heme-containing monooxygenases that are known to influence many biochemical processes. Comparing the cytochrome P450 (CYP) genes in these two species will provide more insight into their genetic composition. The study therefore aims to investigate and understand the similarities and differences in their cytochrome P450, which are likely to play a critical role in their adaptation to soil environments and allow them to utilize different organic compounds. The discovered motifs were identified by using the Multiple Expectation Maximization for Motif Elicitation (MEME) suite, while the intron and exon organization of each CYP gene was analyzed by the Gene Structure Display Server (GSDS). The subcellular localization of the CYPs was predicted by DEEP LOC 2.0, while the evolutionary relatedness of the CYP genes was determined by MEGA X. The result indicated that identified motifs in the genome of *C. curvatus* were more conserved than in *C. terricola*. Similarly, the gene structure study showed that *C. terricola* had more introns than *C. curvatus*. Subcellular localization predictions revealed that most CYPs in both species are localized in the endoplasmic reticulum, emphasizing their crucial roles in lipid metabolism and protein synthesis. Phylogenetic analysis revealed the clustering of CYPs into distinct clades, both species-specific and shared evolutionary relationships, underscoring the genetic diversity and evolutionary significance within these species. Therefore, the observed differences in the structural organization and localization of CYPs between these two species may be strongly associated with the environmental adaptations, protein composition, and control of gene expression. This study, therefore, contributes to our understanding of the relationship between fungal genomes' organization and environmental adaptation. It also lays the foundation for future exploration of fungal capabilities across different habitats and for application in biotechnology.

**Keywords:** Comparative, *Cryptococcus terricola, Cryptococcus curvatus,* Cytochrome P450, Motif.

## INTRODUCTION

The genus *Cryptococcus* includes over 50 species of encapsulated yeast, several of which are pathogenic to humans and animals. *Cryptococcus neoformans* and *Cryptococcus gattii* are the two primary pathogenic species that cause *cryptococcosis,* a potentially fatal disease affecting immunocompromised individuals. *Cryptococcus terricola* and *Cryptococcus curvatus* are yeasts with a wide range of ecological niches, including soil, plants, and insects (Hagen *et al*., 2015; Sreenivasaprasad *et al.,* 2018). Both species are known to produce a wide range of secondary metabolites with potential pharmaceutical and industrial applications (Chen *et al.,* 2019).

*Cryptococcus terricola* is an oleaginous yeast strain capable of directly assimilating starch as a carbon source for the consolidated bioprocessing of hydrocarbon products (Chen *et al.,* 2019).Lipid production in *Cryptococcus terricola* favors the production of unsaturated 18-carbon chain-length fatty acids, with additional minor contributions of saturated 18-carbon and 16-carbon fatty acids (Sreenivasaprasad *et al.,* 2018). *Cryptococcus curvatus,* on the other hand, is also an oleaginous yeast strain, but its capability is in the assimilation of xylose, lactose, glucose, and sucrose, as well as a variety of agricultural and food processing wastes, as carbon sources (Podobnik *et al*., 2017).

One of the problems of food and agricultural bioprocessing industries is dealing with waste generated during such processes; untreated waste constitutes a threat to the environment by adding to the challenge of climate change. Wastewater from food plants and other starchy wastes, including cassava pulp, is usually thrown away. A significant amount of cassava pulp (about 552,000 tons annually) is wasted (Tanimura et al., 2014). Cassava pulp is a byproduct of starch manufacturing in tropical countries and comprises 50–60% residual starch on a dry weight basis. High levels of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) are seen in starchy wastewater, which poses major environmental risks. According to reports, processing one ton of cassava generates at least 0.60 $m^3$ of wastewater (Tanimura et al., 2014). Nonetheless, the manufacturing of biolipids may benefit greatly from this wastewater, and *C. terriolola,* because of its high lipid-accumulating ability, is a candidate organism for converting this waste with great risk to the environment into biofuel (Tanimura et al., 2014).

Cytochrome P450 (CYP) enzymes comprise a large superfamily of heme-containing monooxygenases that play a crucial role in a wide range of metabolic processes, including drug metabolism, xenobiotic detoxification, and biosynthesis of secondary metabolites (Dauda et al., 2023). These CYP genes have been identified in a variety of organisms, including fungi (Dauda et al., 2022a; Córdova et al., 2017).

In fungi, CYP enzymes are involved in the biosynthesis of secondary metabolites, including mycotoxins, pigments, and antibiotics. Several studies have demonstrated that CYP enzymes are also involved in the metabolism of xenobiotics, including drugs and environmental pollutants (Podobnik *et al*., 2017; Dauda et al., 2023).

Bioremediation is a process that utilizes biological systems, including microorganisms, to degrade or detoxify pollutants in the environment (Dauda et al., 2022b). Cytochrome P450 enzymes play a crucial role in the biodegradation of many toxic chemicals, including polycyclic aromatic hydrocarbons (PAHs), pesticides, and herbicides (Peng *et al*., 2020).

*C. curvatus* has been reported to overcome one of the major challenges in fermentation of hemicellulosic streams by utilizing pentoses such as xylose, which are not consumed by the ethanol-producing yeast strain, and hexoses to produce lipids (Samavi et al., 2019).Also, *C. curvatus* was found to be the most efficient of the twelve yeast strains that have been reported to have the ability to co-ferment a sugar mixture including glucose, xylose, and L-arabinose into producing the highest amount of biomass and lipid with concentrations of 17.2 g/L and 5.8 g/L, respectively, on non-detoxified liquid hydrolysate (Samavi et al., 2019).

Similarly, it has been established that oleaginous yeasts produce biolipids, such as triacylglycerol, which are among the most efficient raw materials for the manufacture of biodiesel (Tanimura et al., 2014). The ability to create lipids from organic waste materials that contain different kinds of polysaccharides, such as cellulose and starch, is one benefit of using oleaginous yeast for lipid production.(Tanimura et al., 2014)

Therefore, the ability of these organisms to convert agricultural and food processing waste into useful biochemicals and biofuels even in the presence of non-detoxified liquid must be a function of special genetic makeup. One of the challenges in gene manipulation for biotechnological applications is the lack of information on the genetic makeup and the basis underlying differences in metabolism and biotransformation of xenobiotic compounds. Despite being closely related species, *C. curvatus* and *C. terricola* exhibit diverse metabolic capacities and responses to environmental stress, which would be attributed to variations in their cytochrome p450 (CYP) gene repertoire and regulation. Therefore, it is important to investigate and compare the CYP gene diversity, localization, organization, and functional properties between *Cryptococcus terricola* and *Cryptococcus curvatus* for potential application in bioremediation and biotechnology, as well as to gain insights into their evolutionary and ecological significance.

The study therefore aims to investigate and understand the similarities and differences in the cytochrome P450 of *C. terricola* and *C. curvatus,* which are likely to play a critical role in their adaptation to soil environments by allowing them to utilize different organic compounds and

cope with the presence of toxic substances even during biochemical synthesis (Chen and Yang 2020).

## MATERIAL AND METHODS

### Selection of *Cryptococcus* species

Two (2) *Cryptococcus* species were selected for the present study based on the similarity in the number of CYP sequences in the Mycocosm. *C. terricola JCM 24523 v1.0* and *C. curvatus ATCC 20509 v1.0.* The protein gene sequences of the two *Cryptococcus* species were retrieved from the Joint Genome Institute, out of which a total of 36 were considered valid for further analysis in the present study after screening them for the presence of the CYP450 conserved domain in the NCBI batch CD database (Dauda *et al.,* 2022).

### Sequence Retrieval

Protein, coding, and genomic sequences of Cytochrome P450 of *C. terricola JCM 24523 v1.0* and *C. curvatus ATCC 20509 v1.0* were downloaded from the Joint Genome Institute (JGI) fungal genome database, Mycocosm, publicly available at (https://mycocosm.jgi.doe.gov/mycocosm/home). The genomic sequences were deposited by Close and Ojumu (2016) and Close et al. (2016).

### Phylogenetic Analysis

This analysis was performed using MEGA 11 software (Tamura *et al.,* 2021), in which multiple sequence alignment was carried out using the MUSCLE algorithm with all parameters set at default (gap open: -2.9, gap extend: 0.00, hydrophobicity multiplier: 1.20, maximum iteration: 16, clustering method: UPGMA, and min diag length: 24). Involving 36 protein sequences from the two selected species. The phylogenetic tree was constructed by the maximum likelihood algorithm as earlier described by Dauda *et al.,* 2022.

### Subcellular Localization

The subcellular localization of CYPs in *C. terricola* and *C. curvatus* was predicted using an online web server (DeepLoc 2.0) (https://services.healthtech.dtu.dk/service.php?DeepLoc-2.0 for predicting subcellular localization of eukaryotic proteins.

### Identification of motifs

The discovered motifs of the cytochrome P450 gene in *C. terricola* and *C. curvatus* were identified by an online server, Multiple Expectation Maximization for Motif Elicitation (MEME) suite, freely available at https://meme-suite.org/meme/ using genomic sequence (Bailey *et al.,* 2009). CYP sequences from each of the selected species were submitted using the default parameters (classic mode, zero or one occurrence per sequence for the site distribution, and the number of discovered motifs was set at 10). The number of motif counts was set at 10; the minimum width of the motif was set at 6 amino acids, while the maximum was 50 amino acids.

### Gene Structure Analysis

Gene Structure Display Server (GSDS 2.0), an online web server freely available at https://gsds.gao-lab.org/, was used to determine the intron and exon arrangement on each of the cytochrome P450 genes in *C. terricola* and *C. curvatus.*The server graphically displayed the introns and exons after loading the coding and genomic FASTA sequences of *C. terricola* and *C. curvatus*.

## RESULTS

### Phylogenetic Analysis

The results obtained from the phylogenetic analysis, shown in Figure 1, revealed that thirty-six (36) protein sequences were divided into five (5) clades differentiated by distinct colours. Twelve (12) protein sequences were clustered in clade I. Among the protein sequences that were clustered in clade 1, *C. curvatus* was found to be dominant, with ten (10) protein sequences. Seven (7) proteins were clustered in clade II, and the protein sequence of *C. terricola* was found to be present in all the clades. Six (6) protein sequences were clustered in clade III. Among the six (6) protein sequences, four (4) protein sequences belong to *C. terricola*, while two (2) protein sequences belong to *C. curvatus,* making *C. terricola* dominant in clade III. Two (2) protein sequences were clustered in clade IV, and they both belong to *C. curvatus.* Nine (9) protein sequences were clustered in clade V, in which seven (7) protein sequences belong to *C. terricola* and two (2) protein sequences belong to *C. curvatus.*The phylogeny was inferred using the Maximum Likelihood method and the Jones-Taylor-Thornton model of amino acid substitutions, and the tree with the highest log likelihood (-47,502.95) is shown. The percentage of replicate trees in which the associated taxa clustered together (50 replicates) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. The analytical procedure encompassed 36 amino acid sequences with 1,375 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

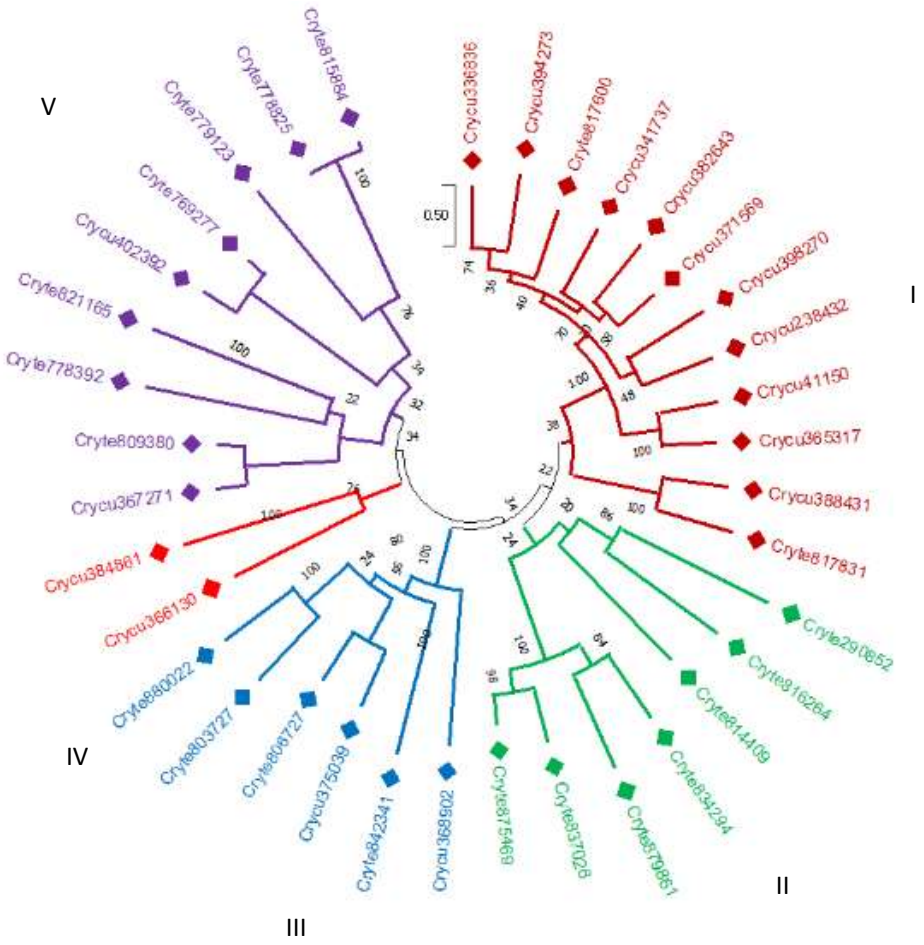


**Figure 1**. Evolutionary relationship of cytochrome P450 proteins in *C. terricola* and *C. curvatus*.

**Subcellular Localization Prediction**

The subcellular localizations of CYPs in *Cryptococcus curvatus,* as illustrated in Figure 2, have shown that they were majorly localized in the endoplasmic reticulum (15 out of 16 CYPs, representing 93.75%), and 1 CYP was found to be localized in the cytoplasm (representing 6.25%). Also, in *Cryptococcus terricola,* the CYPs were majorly localized in the endoplasmic reticulum (13 out of 20 CYPs, representing 65%), while 3 out of 20 CYPs, representing 15%, were localized in the cytoplasm, and 4 out of 20 CYPs (representing 20%) were localized in the mitochondria, while none were found to be localized in the mitochondria of C. *C.curvatus*.

**Motif Identification on the CYP Genes in the Genome of *C. curvatus***

The results, as shown in Figure 3a, revealed that all ten (10) discovered motifs were found to be conserved in five (5) genes: *Crycu336836*, *Crycu341737, Crycu394273, Crycu398270*, and *Crycu371569*. The result also revealed that there are nine (9) discovered motifs in four (4) CYPs (*Crycu41150, Crycu382643, Crycu238432*, and *Crycu365317*). The CYPs with the least occurring motifs are *Crycu366130* and *Crycu402392,* with three (3) discovered motifs each. Motifs 1, 2, and 8 are conserved in all sixteen (16) CYPs in *C. curvatus.* The discovered motifs, symbols, and sequence logo of the predicted motifs in *C. curvatus* are shown in Figures 3b and 3c, respectively. In *C. curvatus,* motifs 3 and 4 are the widest, with a width of fifty (50) (Figure 3c).

The most conserved motifs are motifs 1, 2, and 8, which are found to be present in all 16 CYPs (Figure 3b). Motif 1 has a consensus sequence (FXXGXRXCXG). This motif contains amino acid sequences that include phenylalanine (F), glycine (G), arginine (R), and cysteine (C), while the 'X' can be any amino acid residue. Motif 2 has a consensus sequence of (AGXDTT), as can be seen in Figure 3c. The amino acid composition includes

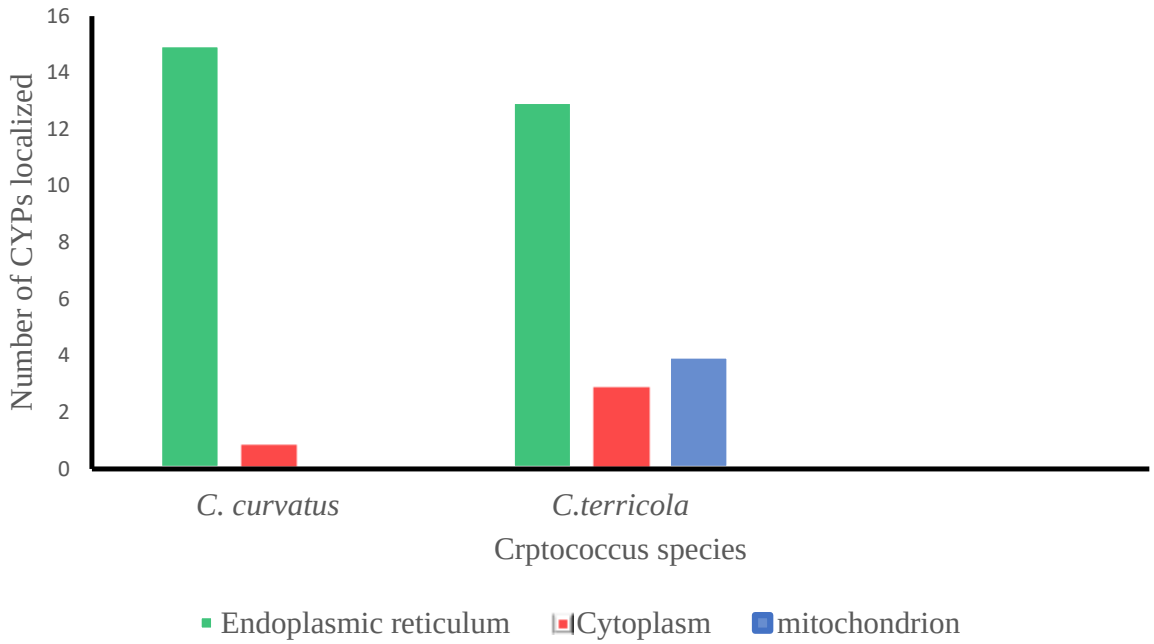


**Figure 2.** Predicted Subcellular localization of CYPs in *C. terricola* and *C. Curvatus*.

alanine or glycine (A/G), any amino acid residue (X), aspartate (D), and a pair of threonine residues (TT).

**Motif Identification and conservation on the CYP genes in the genome of *C. terricola***

The result, as seen in Figure 4a, motifs two (2) and four (4) were found to be conserved in all the CYP genes in *C. terricola*. Motifs one (1) and three (3) were conserved in nineteen (19) and eighteen (18) CYPs, respectively. More so, the motifs with the least conservation are motifs ten (10) and eight (8), which were present in just two (2) and three (3) CYPs, respectively. None of the twenty (20) CYPs in *C. terricola* have all ten (10) discovered motifs. Furthermore, eight (8) motifs were found to be conserved in three CYPs (*Cryte875469, Cryte837026*, and *Cryte834297*). The CYP with the least number of discovered motifs is *Cryte821165* with three (3) motifs.

In *C. terricola*, motifs 1, 2, and 4 are found to be conserved in all 20 CYPs except for motif 1, which is present in 19 of the CYPs, as seen in Figure 4a. On the sequence logo of the ten discovered motifs in *C. terricola* (Figure 4c), four (4) motifs (6,7,8, and 10) are shown to be the widest with a width of fifty (50). Also, none of the CYP genes have all 10 discovered motifs.

Motif 1 is also the heme-binding motif (FXXGXRXCXG); motif 2 is the ExxR motif with the composition of amino acids glutamate (E) and arginine (R), Motif 4 is the PERW with key amino acid composition that includes Proline (P), Glutamate (E), and Arginine (R). The details are shown in Figure 4c.

**Gene structure analysis of CYP genes in the genome of *Cryptococcus curvatus***

The results of exon-intron structures of cytochrome P450 genes in the genome of *C. curvatus* are shown in Figure 5a. All the genes have a minimum of one and a maximum of eight introns, except for *Crycu385120,* which has no introns. *Crycu388690* is the longest, with only two exons, spanning approximately 4000 bp. *Crycu41409* has the highest number of introns (8). *Crycu238691, Crycu367530,* and *Crycu398529* each have three introns; *Crycu402651* and *Crycu394532* each have two introns. Six CYPs (*Crycu388690, Crycu375298, Crycu369161, Crycu366389, Crycu341996*, and *Crycu337095*) are found to have only a single intron.

**Gene Structure Analysis of CYP Genes in *C. terricola***

All the CYPs in *C. terricola* have a minimum of three and a maximum of fifteen introns, as shown in Figure 5b. Cryte 879924, 837089, and 834357 have the highest number of introns (15), while Cryte 880085 has the lowest number of introns. All the genes have no untranslated regions (UTR).

**Gene structural analysis of *C. curvatus and C. terricola***

All the genes have a minimum of one and a maximum of eight introns in *C. curvatus,* while the genes in *C. terricola*

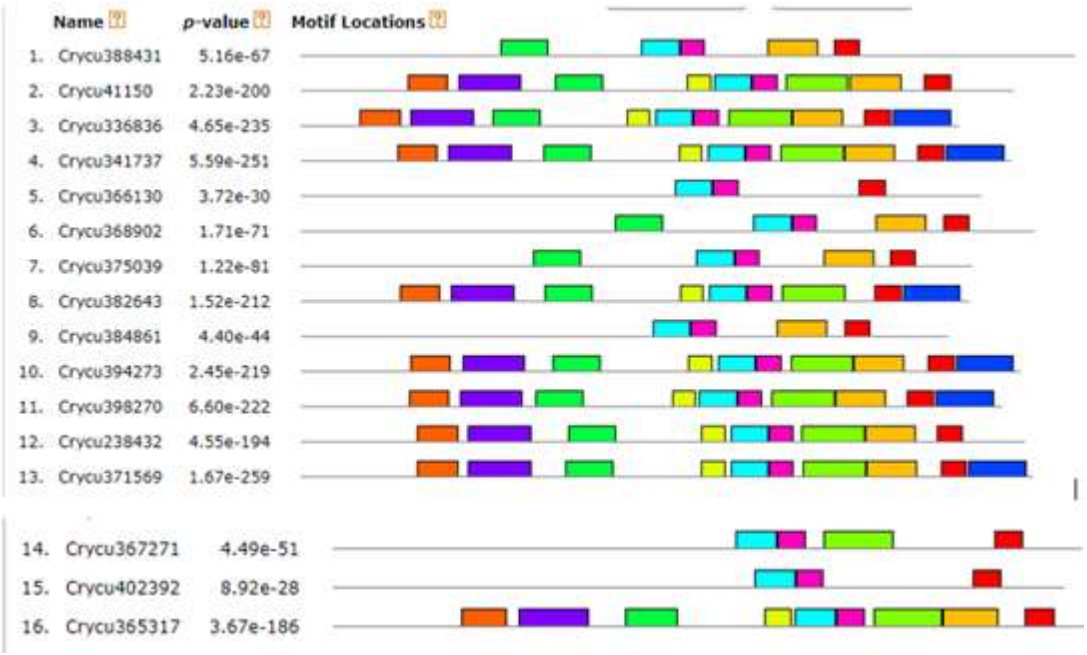


**Figure 3a.** Predicted motifs in *Cryptococcus Curvatus.*

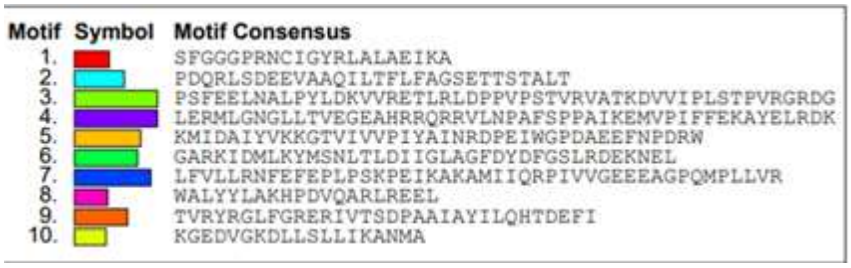


**Figure 3b**. Motif symbol of identified CYPs in *C. curvatus.*

have a minimum of three and a maximum of fifteen introns. *C. curvatus* has a gene with no introns, while all the genes in *C. terricola* have introns.

## DISCUSSION

### Phylogenetic tree

The phylogenetic analysis revealed the evolutionary relationships among the CYP proteins of *C. terricola* and *C. curvatus*. The proteins clustered into five distinct clades, with varying levels of representation from each species, suggesting a closer evolutionary relationship between the species.

This phylogenetic relationship reflects the shared evolutionary history of these two species and their divergence into distinct clades. The presence of CYP proteins from both species within some clades suggests potential functional similarities or conserved roles in specific metabolic pathways. The CYP genes of *C. terricola* and *C. curvatus* share clades, indicating a monophyletic origin and the persistence of specific genetic traits over time. It also implies that these genes might carry out comparable biochemical processes, like metabolizing comparable substrates, which could be essential for the organism's adaptation and survival (Ortiz-Álvarez et al., 2024).

The fact that C. curvatus in clade I and *C. terricola* in clade III are observed to be dominant over one another suggests that these CYPs may have experienced rapid gene duplication events, resulting in a large number of CYP genes. According to Ortiz-Álvarez et al. (2024), this may be a reaction to a variety of ecological opportunities


**Figure 3c.** Sequence logo of ten predicted motifs in the Cytochrome P450 of *Cryptococcus curvatus*. The x-axis displays the type and location of amino acids. The amino acid stacks are displayed on the y-axis. The individual height of each symbol specifies the relative frequency of a nucleotide base at a site within an amino acid stack, whereas the total height of the stacks indicates the sequence conservation at that position.

or challenges.

Clade dominance can result from CYP gene proliferation in a species due to certain ecological niches or physiological needs (Pankov et al., 2021).To survive in its environment, a species may have evolved specialized roles or enhanced metabolic capacities, which could explain its dominance in a clade (Pankov et al., 2021).

**Subcellular Localization**

In fungi, cytochrome P450 (CYP) enzymes are known to perform a variety of metabolic functions, including the production of significant metabolites and the detoxification of xenobiotics. These enzymes are primarily found in the endoplasmic reticulum (ER) (Dauda et al., 2023) and, to a lesser degree, in mitochondria, as observed in both *C. curvatus* and *C. terricola*. The metabolic processes and functional roles that these enzymes exhibit are influenced by their localization within the cell (Skellam, 2022); for example, the CYP enzymes PatH and PatI are located in the ER during the synthesis of patulin, a mycotoxin produced by Penicillium species. Their interaction with other cytosolic enzymes in the route is facilitated by this location, which speeds up the biosynthesis process (Skellam, 2022). The structure of the ER membrane ensures that the active sites of CYP enzymes are available for substrate metabolism, supporting the enzymes' correct orientation and activity. Additionally, by facilitating effective coupling with essential redox partners, this strategic location maximizes the catalytic effectiveness of these enzymes (Boenisch et al., 2017).

Similarly, the localization of some CYPs in the mitochondria is seen in *C. terricola*. Suggest involvement in essential metabolic functions, such as sterol production and the metabolism of other endogenous substances. The ability of these enzymes to directly affect mitochondrial function and energy metabolism, due to their localization within mitochondria, is essential for fungal growth and adaptability. This distribution may reflect the diverse metabolic capabilities between the two species.

**Motif Identification and Conservation**

This study revealed the presence of conserved motifs within the CYP genes of both *Cryptococcus terricola* and *Cryptococcus curvatus*. In *C. curvatus*, five CYP genes were found to contain all ten conserved motifs, highlighting the conservation of these sequences in this species. In contrast, none of the CYP genes in *C terricola* contained all ten discovered motifs, suggesting a.

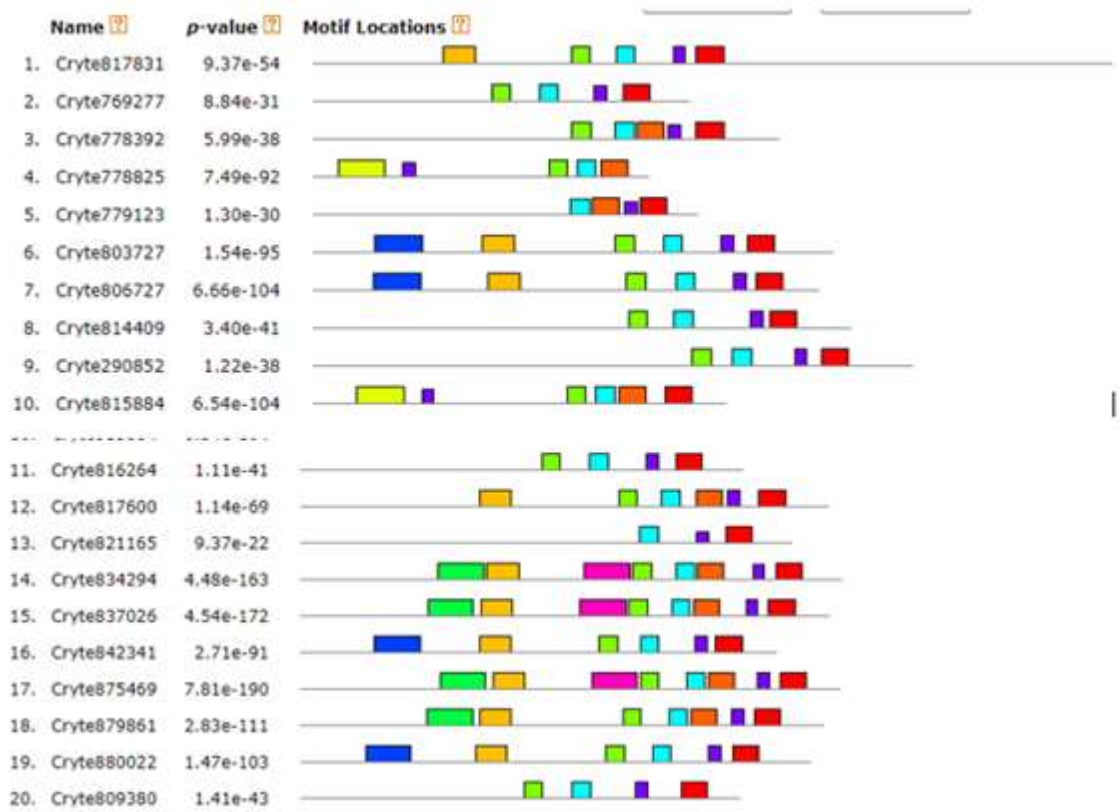


**Figure 4a.** Predicted motifs in *Cryptococcus terricola*.

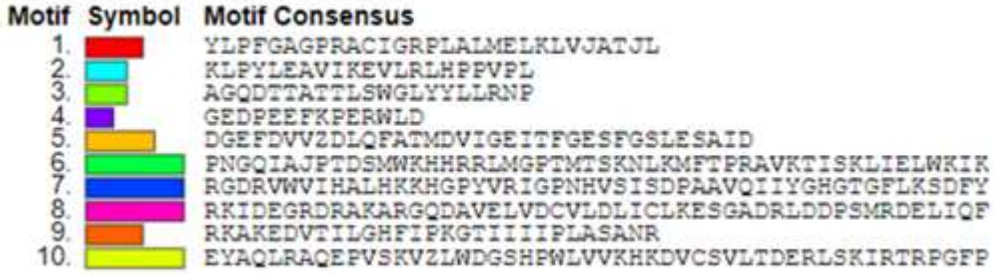


**Figure 4b.** Motif symbol of the CYPs in *Cryptococcus terricola.*

higher degree of variation in this species.

In *C. curvatus,* the most conserved motifs are motifs 1, 2, and 8, which are found to be present in all 16 CYPs. Motif 1 is the heme-binding motif (FXXGXRXCXG), which is one of the characteristic features of cytochrome P450. This motif contains amino acid sequences that include phenylalanine (F), glycine (G), arginine (R), and cysteine (C), while the 'X' can be any amino acid residue. This motif is essential for heme group anchoring and is universally conserved throughout P450 enzymes. The invariant cysteine residue ligates the iron atom at the core of the heme, a crucial contact necessary for the monooxygenase activity of the enzyme. Effective electron transport and substrate oxidation depend on this exact heme placement (Guengerich, 2018; Shaik et al., 2020).

Motif 2 is the oxygen-binding motif with a consensus sequence of AGXDTT, as can be seen in Figure 3c. The amino acid composition includes alanine or glycine (A/G), any amino acid residue (X), aspartate (D), and a pair of threonine residues (TT). This motif is crucial for the binding and activation of molecular oxygen. The conserved threonine or threonines are thought to

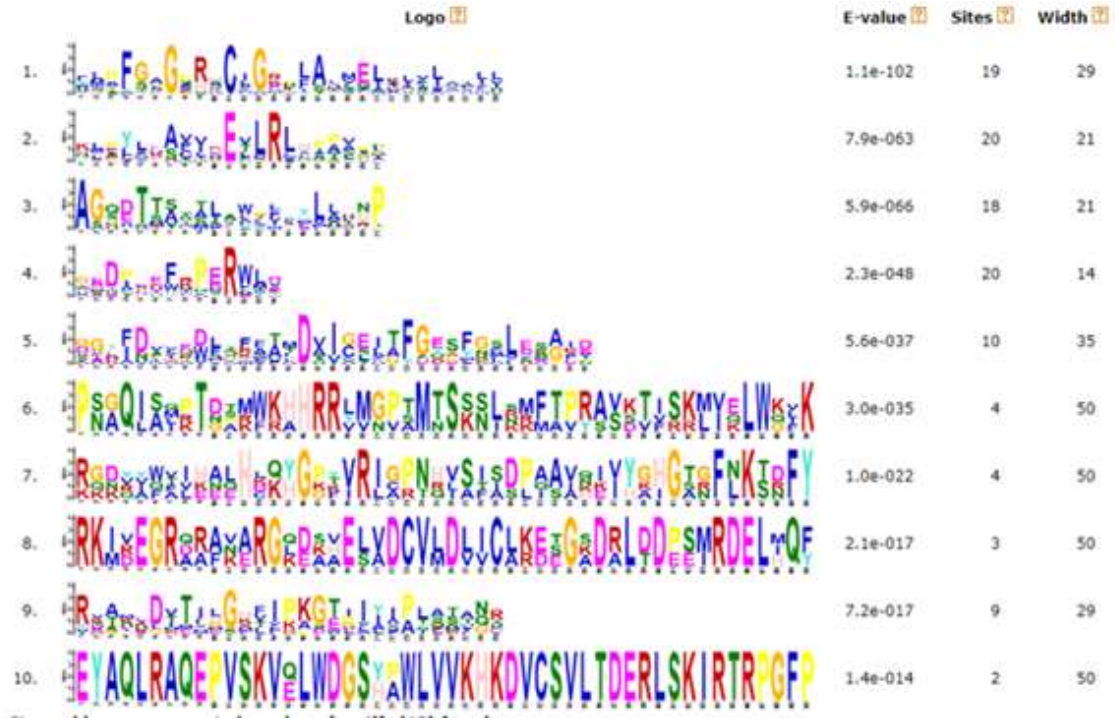


**Figure 4c.** Sequence logo of ten predicted motifs in the Cytochrome P450 of *Cryptococcus terricola*. The x-axis displays the type and location of amino acids. The amino acid stacks are displayed on the y-axis. The relative frequency of a nucleotide base at a site is specified by the individual height of each symbol within an amino acid stack, whereas the total height of the stacks indicates the sequence conservation at that position.


**Figure 5a.** Exon-intron structures of cytochromep450 gene in *C. curvatus*.

contribute to proton transport throughout the catalytic cycle, which facilitates the creation of reactive oxygen species necessary for substrate oxidation (Munro et al., 2018; Shaik et al., 2020).

In *C. terricola*, motifs 1, 2, and 4 are found to be conserved in all 20 CYPs except for motif 1, which is present in 19 of the CYPs. Motif 1 is also the heme-binding motif (FXXGXRXCXG); motif 2 is the ExxR motif

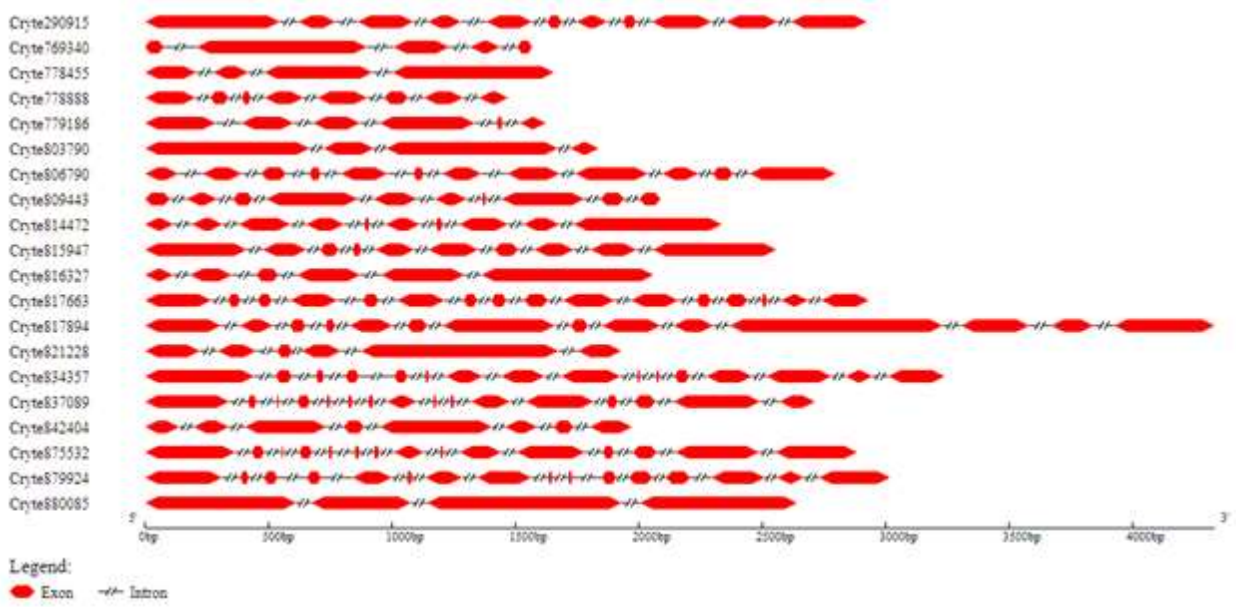


**Figure 5b**. Exon-intron structures of cytochromep450 gene in *C. terricola*.

with the composition of amino acids glutamate (E) and arginine (R); these key amino acids, which are charged residues, can form salt bridges and hydrogen bonds. This motif is recognized to contribute to maintaining the overall architecture of the enzyme. For catalytic efficiency, the interactions between the charged side chains guarantee correct folding and preserve the integrity of the heme-binding pocket. (Guengerich, 2018; Koch & Sligar, 2020). Motif 4 is the PERW with a key amino acid composition that includes proline (P), glutamate (E), and arginine (R), which contribute to polar interactions, and phenylalanine (F), which provides hydrophobic character. It is believed that the PERF motif facilitates the proper alignment of secondary structure elements adjacent to the heme-binding region, which is essential for maintaining the active site's structural integrity and optimizing electron transfer pathways during catalysis (Munro et al., 2018; Shaik et al., 2020). The conservation of the heme-binding motif (FXXGXRXCXG) in the CYPs of both C. *curvatus* and *C. terricola* agrees with other experiments that have reported the conservation of the heme-binding domain (FXXGXRXCXG) as a major characteristic domain of CYPs in fungi, as seen in *Xanthophyllomyces dendrorhous* (Córdova et al., 2017). This conservation has also been reported in prokaryotes, *Arabidopsis thaliana, Homo sapiens,* and other fungi (Chen et al., 2014). The observed variations in the conservation of unique motifs between these two species may be the reason for the wide substrate utilization of *C. curvatus* over *C. terricola*. Also, it may explain substrate specificity and the special ability of *C. curvatus* to utilize substrates even on non-detoxified liquid hydrolysate (Samavi et al., 2019). Fungal CYPs are known to use their conserved motifs in preserving the structure of their catalytic site and also to improve their substrate specificity (Ortiz-Álvarez et al., 2024).

**Gene Structure Analysis**

The gene structure analysis of CYP genes in both species revealed differences in the number of introns. *C. curvatus* exhibited a range of one to eight introns, with one gene having no introns, while *C. terricola* showed a wider range of three to fifteen introns. This variation in intron numbers may reflect differences in the genetic makeup and complexity of these two species.

The number of introns can impact gene regulation, alternative splicing, and protein diversity (Hibbett *et al.,* 2014). In *C. terricola*, with a higher number of introns, the potential for alternative splicing and the production of diverse mRNA isoforms may be greater. This could result in a broader range of protein functions and adaptations to different environmental conditions. In contrast, *C. curvatus*, with fewer introns, may have a simpler gene regulation system, which could affect its adaptability to environmental changes. These differences in gene structure highlight the genetic divergence between the two species and may contribute to their distinct metabolic capabilities and responses to environmental stressors.

Differences in intron numbers between the two fungi species can have several implications, reflecting variations in their genetic makeup and potentially influencing gene expression and regulation.The variations in the number of introns in *C. terricola* and *C.*

*curvatus* indicate evolutionary divergence (Hibbett *et al.,* 2014). Over time, species can accumulate mutations, including changes in intron-exon structures, leading to differences in the number of introns in homologous genes. Moreover, *C. terricola* has more introns and, therefore, has a more complex genomic organization. In contrast, *C. curvatus* has fewer introns, which suggests it might have undergone genome simplification. These variations can contribute to differences in the complexity of gene regulation and expression.

Additionally, intron numbers can impact gene regulation and expression patterns (Girardini et al., 2023). In the case of *C. terricola,* which has more introns, this allows for greater flexibility in alternative splicing, resulting in multiple mRNA isoforms with potentially diverse functions. Protein Diversity (Alternative splicing, influenced by intron-exon structures, can lead to the production of different protein isoforms (Brett *et al.,* 2015). Varied intron numbers may result in species-specific protein diversity, enabling adaptations to different environmental or physiological conditions.

Furthermore, intron sequences can contain regulatory elements that influence gene expression (Maria *et al.,* 2009). Variations in intron numbers may affect the presence and arrangement of these regulatory elements, potentially impacting the timing and level of gene expression in different species. Variations arising from the number of introns across the other species could be due to genomic rearrangements such as insertions or deletions of genomic segments (Burkhard *et al.,* 2006). Various evolutionary forces, including transposon activity or environmental pressures, may drive these rearrangements. Also, the expansion or contraction of gene families in a particular species can influence the number of introns in that species. Finally, gene duplication and subsequent divergence can lead to changes in intron-exon structures among paralogous genes (Aoife *et al.,* 2000).

## CONCLUSION

The study provides a comprehensive analysis of CYP genes in *Cryptococcus terricola* and *Cryptococcus curvatus*. *C. terricola* shows more complex genomic organization in terms of the organization of introns and exons than *C. curvatus,* potentially explaining its substrate specificity for starch rather than its *curvatus* counterpart, which has wider substrate utilization that includes xylose, glucose, and L-arabinose. Moreover, Future research can delve deeper into the functional characterization of conserved motifs and the regulatory mechanisms governing CYP gene expression in these species. This could help in advancing our understanding of their biology and biotechnological potential. Also, validation of the predicted subcellular localization of the CYP genes is recommended.

## LIMITATION

This is an *in silico* study; therefore, experimental validation is required to confirm the predictions of subcellular localizations.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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