Genome-Wide Analysis of Cytochrome P450s in *Ustilago* Species: Annotation and Evolutionary Relationships

W.P. Dauda[1]*, U.J. Ifeanyi[2], P. Abraham[3], C.O. Adetunji[4], E. Glen[5], D. Morumda[6], I. O. Ogra[7] G.W. Peter[8], S.E. Abraham[9], C. Chukwu[3], N.J. Dasoem[6], M.I. Luka[5] and M.P. Abraham[3]

(1) Department of Agronomy, Federal University Gashua, Yobe State, Nigeria; (2) Department of Crop Science, University of Uyo, Uyo P.M.B. 1071, Akwa Ibom State, Nigeria; (3) Department of Horticultural Technology, Federal College of Horticulture, PMB 108, Dadin Kowa, Gombe, Nigeria; (4) Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Iyamho, PMB 04, Auchi, Edo State, Nigeria; (5) Department of Biochemistry, Federal University Lokoja, Lokoja P.M.B. 1154, Kogi State, Nigeria; (6) Department of Microbiology, Federal University Wukari, Wukari P.M.B. 1020, Taraba State, Nigeria; (7) UNESCO International Centre of Biotechnology Nsukka, Nigeria; (8) Department of Biochemistry, Ahmadu Bello University, Zaria 800001, Kaduna State, Nigeria; (9) Department of Agronomy, Bayero University Kano, Kano P.M.B. 3011, Kano State, Nigeria.

*Email address of the corresponding author: wadzanidaudap@fugashua.edu.ng

Abstract

Dauda, W.P., I.J. Ifeanyi, P. Abraham, C.O. Adetunji, E. Glen, D. Morumda, I.O. Ogra, G.W. Peter, S.E. Abraham, C. Chukwu, N.J. Dasoem, M.I. Luka and M.P. Abraham. 2025. Genome-Wide Analysis of Cytochrome P450s in *Ustilago* Species: Annotation and Evolutionary Relationships. Arab Journal of Plant Protection, 43(2): 268-275. https://doi.org/10.22268/AJPP-001304

Cytochrome P450s (Cyp) belong to the superfamily of hemeprotein monooxygenases that metabolize some essential compounds that could support the life of most organisms. Among fungi, *Ustilago* species are of significant economic importance, evidently because they are known to cause substantial agricultural losses globally. The study was conducted to establish the profile, diversity, evolutionary relationship, and family and clan classification of Cyp genes of four *Ustilago* species (*U. bullata, U. maydis, U. hordei* and *U. virens*). A total of 77 Cyp protein genes from the mentioned *Ustilago* species were used in this study. The Cyp genes were clustered into five monophyletic clades. A total of 26 Cyp clans and 35 Cyp families were observed. The distribution of families across *Ustilago* species were: *U. virens* (20) Cyp families, *U. maydis* 521 v2.0 (13) Cyp families, *U. hordei* Uh4857_4 (10) CYP families and *U. bullata* UB2112 (12) Cyp families. The Cyp families across *Ustilago* species were predicted to be associated with primary, secondary and xenobiotic metabolism. Analysis of the subcellular localisations of the cytochrome P450 genes in *Ustilago* species indicated that 88 % of the proteins were confined within the endoplasmic reticulum. The study showed that only 49 Cyp 450 genes from *Ustilago* species are connected with five secondary metabolism-related gene clusters (DMAT, NRPS, NRPS-like, PKS, and PKS-Like), which could valuably be harnessed for the downstream applications and management of these important pathogens. This study could provide relevant background knowledge required in the characterization and the description of the organism's physiology, as well as provide an in-depth insight into the functions of CYPs and diversity research of *Ustilago* species, which could valuably be harnessed for the downstream applications and management of these important pathogens.

Keywords: CYP450, diversity, phylogenetic relatedness, protein genes, *Ustilago*.

Introduction

Ustilaginaceae is a family of smut fungi belonging to the order Ustilaginales, and class of Ustilaginomycetes, consisting of 17 genera and 607 species (Ullmann *et al.,* 2022). The major distinguishing features in the morphology of these genera are the presence of sori and spores (Al-Maaroof & Saed, 2023; Begerow *et al.,* 2014). *Ustilago* has been reported to survive in hosts of the Poaceae family, which are deficient in soral structure, especially small pillars, spore-like balls, and sterile cells (McTaggart *et al.,* 2012). *Ustilago* species have been reported to be responsible for a huge global agricultural loss (Zarafi & Dauda, 2019; Zuo *et al.,* 2019). They usually attack crops before maturity by producing sori which disintegrates to unleash numerous dark-colored spores (McTaggart *et al.,* 2012). *U. hordei* Uh4857_4 (Pers.) Lagerh., is responsible for the occurrence of covered smut in barley (*Hordeum* spp.) and oats (*Avena* spp.) (Oekmen *et al.,* 2021). *Ustilago virens* cause green smut, a predominant disease of rice, prevalent in rice-producing parts of the world (Singh & Pophaly, 2010). *U. virens* is regarded as a pathogen that damages grain in regions where rice is predominantly cultivated across the globe (Jose *et al.,* 2023).

It has been discovered that *U. maydis* 521 v2.0 can cause corn's common smut or “huitlacoche”. However, it differs from other Ustilaginales, which cause acute epiphytia in important cereal crops (Ruiz-Herrera & León-Ramírez, 2012). These diseases are responsible for colossal losses in crops. The yield depreciation results in chaffiness, a drastic decrease in grain weight, and the unproductiveness of the spikelets surrounding the smut balls (Ruiz-Herrera & León-Ramírez, 2012). Varying crop failures of 0.2 – 49% have been recorded in different regions of the world (Ruiz-Herrera & León-Ramírez, 2012).

Cytochromes P450 consist of an enormous superfamily of hemeprotein monooxygenases that could metabolise diverse essential compounds that could support most organisms’ life which cuts across protists, plants and mammals (Nelson *et al.,* 2004). The CYP is known to

https://doi.org/10.22268/AJPP-001304

perform a vital role in the changes in endogenous and exogenous molecules caused by oxidation (Eccles & Baldwin, 2022; Nelson, 1999). The location and status of CYP450s differ between prokaryotic and eukaryotic cells. In prokaryotic cells, CYP450s are soluble proteins found primarily in the cytoplasm, whereas in eukaryotic cells are predominantly distributed in microsomes, endoplasmic reticulum, and inner mitochondrial membranes. In addition, studies have shown that the CYP450 gene family is characterized by a heme-binding region conserved in its structure (Liu *et al.,* 2022). Cytochrome P450s perform various functions, including the metabolism of steroids, fatty acids, eicosanoids, vitamins A and D, and the metabolism of foreign compounds such as natural products, pharmaceuticals, and carcinogens (Guengerich, 2022). Cytochrome P450 enzymes are capable of catalyzing complex reactions such as regioselective and stereoselective oxidation of deactivated hydrocarbon CH bonds to the corresponding hydroxyl (C-OH) group (Johnston *et al.,* 2011). These P450 enzymes play an important role in the preparatory phase of hydroxylation of fatty acids (Cresnar & Petric, 2011).

Presently, genome-wide analysis of CYP450 supergene family has been carried out in many important fungal species: *Aspergillus nidulans* (Kelly *et al.,* 2009), *Phanerochete chrysosporium* (Syed & Yadav, 2012), *Mycosphaerella graminicola* (Newsome *et al.,* 2013), *Grosmannia clavigera* (Lah *et al.,* 2013), *Trichoderma* species (Chadha *et al.,* 2018); *Fusarium* species (Dauda *et al.,* 2021b), *Aspergillus* species (Dauda *et al.,* 2022a), *Alternaria* spp., (Dauda *et al.,* 2022b), *Candida tropicalis* (Dauda *et al.,* 2022c) *Xylaria* species (Dauda *et al.,* 2023) and *Cryptococcus neoformans* (Dauda *et al.,* 2022d). However, there is still a wide gap in the level of information that exists in the comparative genome-wide study of CYP450s in agriculturally important *Ustilago* species. Hence, this forms a basis and justification for this study. The comprehensive comparative annotations and evolutionary analysis of the cytochrome P450 genes in *Ustilago* species would help to broaden our understanding and provide a foundational basis that could be harnessed for their downstream applications and management.

Materials and Methods

Sequence retrieval and screening of Cyps sequences

The genomes of the four *Ustilago* species, namely: *Ustilago bullata* (syn. *Ustilago bromivora*) UB2112, *Ustilago maydis* 521 v2.0, *Ustilago hordei* Uh4857_4 and *Ustilago virens* (syn. *Ustilaginoidea virens*) were downloaded from the MycoCosm fungal genome database of the Joint Genome Institute. The downloaded genomes were submitted to the NCBI Batch Web CD-search tool to screen for the presence of the CYP P450 conserved domain. Based on the distinctive conserved domains of each protein family, this program classifies and separates putative proteins into various protein families. The results were tabulated, and the potential protein sequences belonging to the P450 superfamily were chosen for subsequent analysis. The EXXR and CXG hallmark motifs of the P450 family, which are present in the chosen proteins, were examined (Syed & Mashele, 2014). The proteins included in this investigation were those that had both motifs, making them legitimate P450s.

Clan and family classification

According to the International P450 Nomenclature Committee's rules, the P450s from the chosen *Ustilago* species were divided into various families and clans (Nelson *et al.,* 2006). Proteins were allocated to the same family as the named homolog P450s have a percentage identity of greater than 40%. Proteins assigned to a new family have a percentage identity of less than 40%. In a similar manner, clans were established utilizing the data's categorization of families into clans.

Phylogenetic analysis of cytochrome P450s

Molecular Evolutionary Genetics Analysis (MEGA X) software was used to conduct ClustalW analysis on the signature domains of P450 for the chosen fungal P450s (Kumar *et al.,* 2018). MEGA-based ClustalW has the benefit of including pairwise alignment and multiple alignments into one ClustalW process. The amino acid patterns in the P450 identity motifs EXXR and CXG were examined in the ClustalW-aligned P450 sequences. The ClustalW algorithm from MEGA was used to choose the amino acid residues for P450 signatures, and the results were generated in tabular form as described by Dauda *et al.* (2021a).

Subcellular localization prediction

The subcellular localization prediction of *U. bullata* UB2112, *U. maydis* 521 v2.0, *U. hordei* Uh4857_4 and *U. virens* CYP450 proteins was performed with the aid of an online server, the DeepLoc 2.0 server. The DeepLoc 2.0 server predicts the multi-label subcellular localization of proteins in eukaryotic organisms using a Neural Networks at its core, an algorithm relying only on sequence information and trained on Uniprot proteins was used (Wang *et al.,* 2021).

Secondary metabolism related gene retrieval

Cytochrome P450s linked with secondary metabolism-related gene groups in *Ustilago* species were retrieved from https://mycocosm.jgi.doe.gov/pages/sm-clusters-summary.jsf? by using the annotation menu after the search for the four *Ustilago* species.

Results

CYP proteins in *Ustilago* species

The results summarized in Table 1 show that a total of 77 Cyp proteins with complete gene sequences in the four queried *Ustilago* species were *U. virens* emerged as the species with the highest number of gene entries (29) followed by *U. maydis* 521 v2.0 (20) while *U. bullata* UB2112 and *U. hordei* Uh4857_4 had the least number of gene entries of 14 each. Four Cyp protein sequences from *U. virens* were found not to have any family match in the database of fungal cytochrome P450 (FCPD).

Cyp450 family and clan diversity and distribution in four *Ustilago* species

The analyzed results on the annotation, abundance and spread of Cyp450 families and clans in four *Ustilago* spp., are presented in Table 1, Figures 1 and 2. Thirty-five Cyp families from twenty-six Clans were identified from the *Ustilago* species. Cyp proteins of *U. virens, U. maydis* 521 v2.0, *U. bullata* UB2112 and *U. hordei* Uh4857_4 were distributed in 18, 14, 10 and 7 clans respectively. *U. virens* had the highest number (22) of Cyp families followed by *U. maydis* 521 v2.0 with 16 Cyp families, whereas *U. hordei* Uh4857_4 recorded the least number (7) of Cyp families. It was observed that only two Cyp families (Cyp51 and Cyp504) were present in all the queried *Ustilago* spp., thereby representing only 5.9% of the total predicted Cyp families. Five Cyp families (Cyp5156, Cyp5026, Cyp5031, Cyp636 and Cyp5028) which is equivalent to 14.7 % of the total cyp families were to be reoccurring in *U. maydis* 521 v2.0, *U. bullata* UB2112 and *U. hordei* Uh4857_4 species while three Cyp families (Cyp5032, Cyp5034 and Cyp5025) representing 8.8 % of the total predicted Cyp families were common in *U. maydis* 521 v2.0 and *U. bullata* UB2112. Interestingly, we found that 50 % (18 Cyp families: Cyp68, Cyp58, Cyp620, Cyp539, Cyp548, Cyp591, Cyp641, Cyp628, Cyp5109, Cyp617, Cyp531, Cyp570, Cyp561, Cyp56, Cyp621, Cyp541, Cyp684, Cyp584) of the total predicted Cyp families were uniquely identified in *U. virens,* whereas 11.8 % (4 Cyp families: Cyp5065, Cyp5027, Cyp5029 and Cyp5030) and 2.9 % (Cyp5033) out of the total predicted Cyp families were only identified in *U. maydis* 521 v2.0 and *U. bullata* UB2112, respectively.

Table 1. Taxonomic distribution of putative cyps in four *Ustilago* species.

Ustilago Species	Genome size (Mb)	Number of predicted genes	Total CYP proteins Sequences	Protein with complete sequences	Family type	Clan type	Sequences with no match in FCPD
Ustilago bullata UB2112	20,447,485	7,185	33	14	12	12	1
Ustilago maydis Uh4857_4	19,664,388	6,785	44	20	16	16	1
Ustilago virens	33,567,624	6,451	49	29	20	16	
Ustilago hordei 521 v2.0	21,150,702	7,110	31	14	6	6	

Clans	CYP5156	CYP550	CYP51	CYP5032	CYP61	CYP5139	CYP53	CYP504	CYP5025	CYP5139	CYP531	CYP5025	CYP530	CYP530	CYP574	CYP627	CYP5031	CYP68	CYP58	CYP533	CYP52	CYP505	CYP548	CYP526	CYP657	CYP574	CYP62	CYP572	CYP547	CYP531	CYP507	Not available	Not available	Not available	Not available
Families	Cyp5156	Cyp636	Cyp51	Cyp5032	Cyp61	Cyp5034	Cyp53	Cyp504	Cyp5026	Cyp5033	Cyp5028	Cyp5025	Cyp5065	Cyp5027	Cyp5029	Cyp5030	Cyp5031	Cyp68	Cyp58	Cyp620	Cyp539	Cyp541	Cyp548	Cyp591	Cyp641	Cyp628	Cyp684	Cyp5109	Cyp617	Cyp531	Cyp570	Cyp561	Cyp584	Cyp56	Cyp621
Ustilago bullata UB2112	1	1	1	1	1	1	1	1	1	1	1	1					1																		
Ustilago maydis Uh4857_4	1	1	1	1	1	1	1	1	2	2	1	2	1	1	1	1	1																		
Ustilago virens				1		1		1	1									2	1	1	3	1	2	1	1	1	1	1	1	1	1	1	1	1	1
Ustilago hordei 521 v2.0	2	1	1						1	3	1						1																		

Figure 1. Cytochrome P450 families identified in four *Ustilago* species.

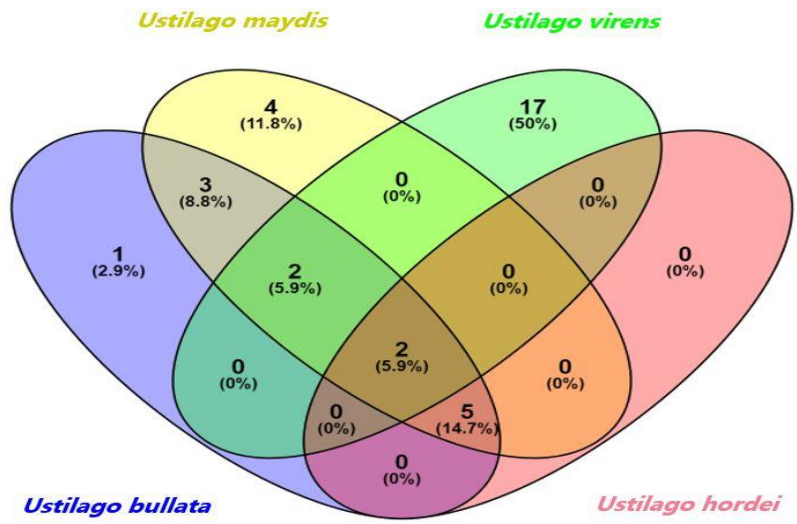

Figure 2. Venn diagram showing the distribution and relatedness of Cyp families among four *Ustilago* species.

Phylogenetic clustering of cytochrome P450 families among four *Ustilago* species

The links between the Cyp families of various *Ustilago* species were better understood through comparisons at the gene level and the identification of P450s. Figure 3 presents a phylogenetic tree showing the evolutionary relationships among *Ustilago* cytochrome P450 proteins. In addition, Table 2 summarizes the placement of various Cyp families and clans in eight phylogenetic classes with estimation capabilities. A total of 77 cytochrome P450 proteins from 4 different *Ustilago* species were divided into 5 phylogenetic classes by evolutionary analysis (Figure 3). The phylogenetic clustering revealed multiple branches, which indicates that there was widespread evolutionary diversity. The diverse Cyps discovered in the four species cluster into five separate phyletic clades. Clade III recorded the highest cyp gene entries (19) from twelve Cyp families (Cyp5029, Cyp628, Cyp5030, Cyp58, Cyp5109, Cyp531, Cyp570, Cyp53, Cyp5028, Cyp684, Cyp561, Cyp548) involved in primary and xenobiotic metabolic functions. This is followed by clades I and V with 17 and 16 Cyp proteins from seven (Cyp51, Cyp61, Cyp5026, Cyp5156, Cyp641, Cyp636 and Cyp68 involved in primary, secondary and xenobiotic metabolism) and six (Cyp61, Cyp56, Cyp539, Cyp584, Cyp5025 and Cyp5026 involved in primary and xenobiotic metabolism) Cyp families, respectively. Clade II had the fewest Cyp protein entries (11) from six Cyp families (Cyp541, Cyp504, Cyp5065, Cyp5027, Cyp621, Cyp620) involved in primary and xenobiotic metabolic functions. With members in three clades, Clan 5026 was highlighted as the largest (I, IV and V). All the phyletic clades were predicted to be actively involved in xenobiotic metabolic processes.

Table 2. Phylogenetic clustering of Cytochrome P450 families and clans among four *Ustilago* species.

Phylogenetic Clade	Sequence entry	CYP Families	CYP Clans	Putative functions
I	17	Cyp51, Cyp61, Cyp5026, Cyp5156, Cyp641, Cyp636, Cyp68	CYP51, CYP61, CYP5025, CYP5156, CYP657, CYP68, CYP550	Primary metabolism, Xenobiotic /Secondary metabolism
II	11	Cyp541, Cyp504, Cyp5065, Cyp5027, Cyp621, Cyp620	CYP505, CYP504, CYP530, CYP533	Primary metabolism, Xenobiotic metabolism
III	19	Cyp5029, Cyp628, Cyp5030, Cyp58, Cyp5109, Cyp531, Cyp570, Cyp53, Cyp5028, Cyp684, Cyp561, Cyp548	CYP627, CYP62, CYP507, CYP58, CYP574, CYP53, CYP531, CYP572, CYP548, CYP56	Primary metabolism, Xenobiotic metabolism
IV	14	Cyp5026, Cyp5034, Cyp5033, Cyp5031, Cyp617, Cyp5032, Cyp591	CYP5026, CYP5139, CYP5031, CYP547, CYP5032, CYP526	Xenobiotic metabolism, Secondary metabolism
V	16	Cyp61, Cyp56, Cyp539, Cyp584, Cyp5025, Cyp5026	CYP61, CYP56, CYP52, CYP5025, CYP5026	Primary metabolism, Xenobiotic metabolism

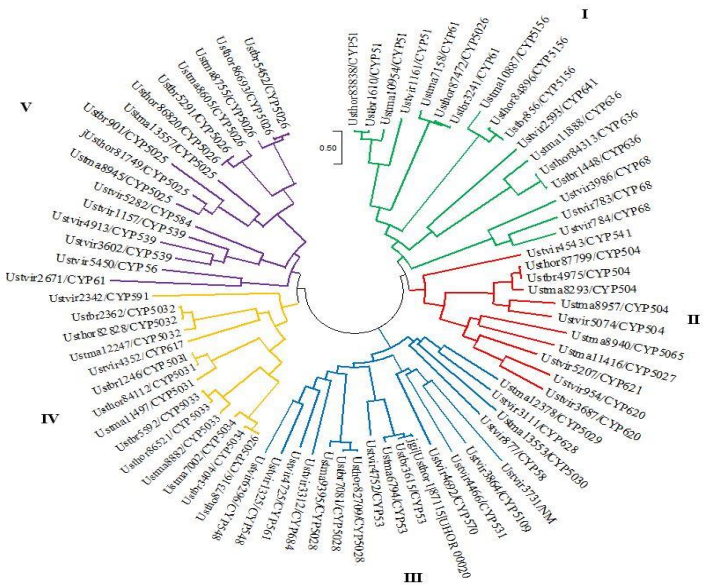

Figure 3. Evolutionary relationships of cytochrome P450 proteins among four *Ustilago* species (*U. bullata* UB2112*, U. virens, U. maydis* 521 V2.0*,* and *U. hordei* Uh4857_4). Phylogenetic tree was constructed using MEGA X software.

Subcellular localization of P450 proteins in *Ustilago* species

The Cyp proteins are predicted to be involved in numerous activities that participate in the xenobiotic metabolism of the genus *Ustilago*. In predicting the potential intracellular localization of the *Ustilago* CYP450 gene, 68 of the total sequences encoded by the *Ustilago* CYP450 genes are confined in the endoplasmic reticulum representing 88% of the CYP450 gene discovered from 4 *Ustilago* species. Additional cellular components were also observed to include CYP450, such as the mitochondria and plastid, which accounted for 5.2% and 1.3% of the proteins encoded by CYP450 genes respectively in *Ustilago* species (Figure 4).

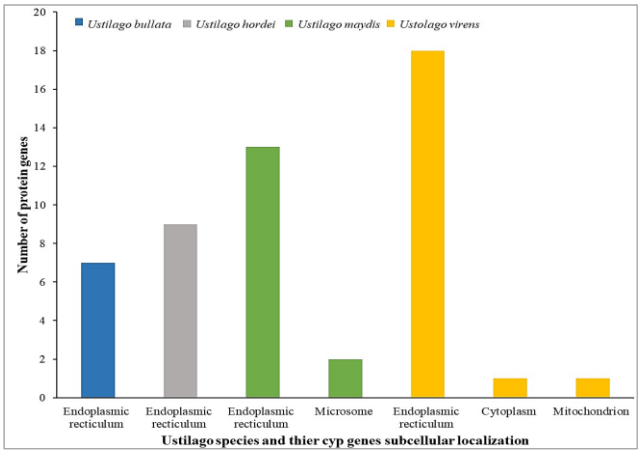

Figure 4. Prediction of subcellular localization analysis of protein genes of four *Ustilago* species.

Identification of cytochrome P450s linked to gene clusters for secondary metabolism

The analysis of cytochrome P450 genes in the four *Ustilago* species revealed that the genes are connected to five (DMAT, NRPS, NRPS-like, PKS, and PKS-Like clusters) secondary metabolic related gene clusters. *U. maydis* 521 v2.0 was discovered to be associated with all the metabolism-related gene clusters [DMAT (2), NRPS (3), NRPS-Like (7), PKS (3), and PKS-Like (3)]. *U. hordei* Uh4857_4 (NRPS (2), NRPS-Like (5), PKS (1), and PKS-Like (1) and *U. virens* (NRPS (4), NRPS-Like (7), PKS (2), and PKS-Like (2) were found to participate in four of the putative secondary metabolism-related gene clusters each while *U. bullata* UB2112 was predicted to be involved in only three [NRPS (2), NRPS-Like (5), and PKS (2)] putative secondary metabolism-related gene clusters (Figure 5).

Discussion

The comparative analysis of Cytochrome P450s in *Ustilago* species has shown the pattern of their diversity, similarities and peculiarities in ancestral evolutionary processes. This study has confirmed the existence of 77 Cytochrome P450 genes in the four *Ustilago* species, and their phylogenetic classification into 5 clades reveals the abundance and relatedness of these Cyps during evolution. Dauda *et al.* (2022a) previously opined that CYPome are dynamic and vary among species of organisms as influenced by their developmental features, feeding and able to thrive within a particular niche in ecology. The occurrence and distribution of 35 and 26 P450 families and clans, respectively in the queried *Ustilago* species suggest their diverse significant roles in *Ustilago* spp. The observed expansion of families and clans of P450s in the queried *Ustilago* species is possible via tandem duplication of Cyp genes in evolution as reported in fungi (Feyereisen, 2011; Sello *et al.,* 2015). Variations in the number of Cyp genes, families and clans have been previously reported among several fungal species (Chadha *et al.,* 2018; Dauda *et al.,* 2021a; Dauda *et al.,* 2022a; 2022b). When compared to other species, *U. virens* emerged as the species with the greatest number of Cyp genes and families. This could be because of several distinctive characteristics like host specialization, pathogenicity, ecological adaption, etc. In addition, the majority of Cyp families are *U. virens* was annotated for the several xenobiotics it metabolized. Cyp51 and Cyp61 proteins have been identified as the most conserved protein genes in the P450 superfamily, with a wide distribution in the kingdom of fungi (Ichinose, 2014; Zhang *et al.,* 2019). The close clustering of Cyp51 and Cyp61, as observed in phylogenetic Clade I, further supported their close association in the evolution and performance of similar putative cellular activities (sterol biosynthesis). This finding was comparable to the report of Chadha *et al.* (2018) on the phylogenetic analysis of *Trichoderma* spp., who also reiterated that Cyp61 emerged as a duplicate of Cyp 51 gene in evolution. However, we observed that Cyp51 and Cyp504 were the only families common to all the queried *Ustilago* species, while Cyp61 and Cyp53 were found in three (*U. bullata* UB2112, *U. maydis* 521 v2.0 and *U. virens*) out of the four *Ustilago* species under study. Cyp53 is conserved and widely spread in the *Basidiomycota* and *Ascomycota* divisions of the fungal kingdom (Ichinose, 2014). Cyp51 protein genes are known to exhibit very specific metabolic activities (Lepesheva & Waterman, 2007; 2011), such as primary metabolism, particularly biosynthesis of sterols (Ichinose, 2014) and regulating virulence in fungal pathogens by elongating their mycelia and increasing invasive growth during pathogenesis (Wu *et al.,* 2018).

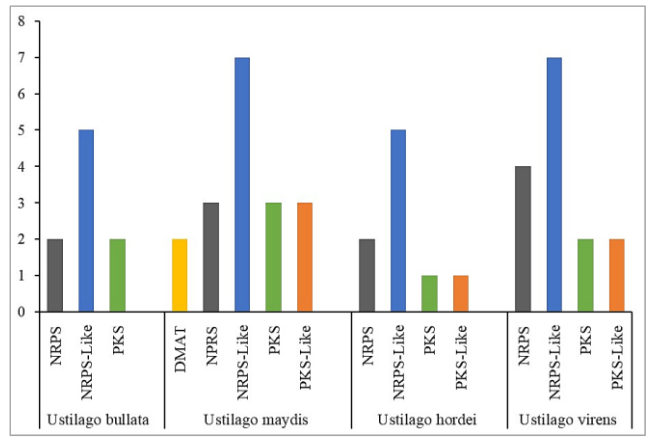

Figure 5. Identification of cytochrome P450 genes in *Ustilago* species associated with secondary metabolism-related gene cluster**.**

The level of pathogenicity observed in the *Ustilago* species to cereal crops could be linked to the presence of Cyp51. More so, Cyp51 protein genes have been a major target in controlling fungal phytopathogens (Ichinose, 2014; Kelly & Kelly, 2013).The expansion of Cyp504 genes to all the queried *Ustilago* spp. elucidate the vital role of these genes in metabolizing various xenobiotics such as fungicides, aiding the resistance of *Ustilago* spp., to fungicide application. The present study revealed that the endoplasmic reticulum (ER) was the major site of subcellular localization for P450s across all the investigated *Ustilago* species. Our finding corroborates previous reports on the subcellular localisation of P450 genes in several fungal species, including *Trichoderma* spp., (Chadha *et al.,* 2018), *Aspergillus* species (Dauda *et al.,* 2022a), *Alternaria* spp., (Dauda *et al.,* 2022b), *Candida tropicalis* (Dauda *et al.,* 2022c), *Fusarium* spp., (Dauda *et al.,* 2021a). Fungi as eukaryotes belong to the Class II enzymatic group engaged in many activities such as the production of mycotoxins, metabolic processes of lipids, phytoalexins and xenobiotics, which are mostly performed in the ER (Werck-Reichhart & Feyereisen, 2000). From our study, most of the Cyp genes families identified in *Ustilago* spp., were found to function in xenobiotic metabolism, an activity reported to be performed in the ER (Etienne & Ingelman-Sundberg, 2008). A total of five secondary related gene clusters (DMAT, NRPS, NRPS-like, PKS, and PKS-Like clusters) were analysed to be encoded by Cyp genes in queried *Ustilago* species. Non-ribosomal peptides, indole terpenes, polyketides synthases, and terpenes are among the common secondary metabolites associated with fungi (Saha *et al.,* 2021). The study revealed the presence and diversity of gene clusters linked to secondary metabolism in *Ustilago* species. and it was established that NRPS-like, NRPS and PKS are the three most prevalent secondary metabolic gene clusters. These numerous gene clusters play a key role in creating the fundamental structure for most secondary metabolites. It has been shown that secondary metabolites contribute to the pathophysiology of many disorders (Al-Maaroof & Saed, 2023; Saha *et al.,* 2021).

الملخص

داودا، و.ب.، إ.ج. إفياني، ب. أبراهام، س.أو. أدتونجي، إ. جلين، د. موروميدا، إ.أو. أوجرا، ج.و. بيتر، س.ف. أبراهام، س. شوكوو، ن.ج. داسوم، إم.آي. لوكا وم.ب. أبراهام. 2025. تحليل مورثات السيتوكروم P450 في كامل مجين أنواع الفطر *Ustilago*: تفسير دورها وعلاقات القرابة فيما بينها. مجلة وقاية النبات العربية، 43(2): 268–275. https://doi.org/10.22268/AJPP-001304

تنتمي مورثات السيتوكروم Cytochrome P450s (Cyp) إلى العائلة الأكبر من الهيموبروتينات أحادية الأوكسيجينات التي تخلِّق بعض المركبات الأساسية التي يمكن أن تدعم معظم الكائنات الحية. من بين الفطور، تتسم أنواع الجنس *Ustilago* بأهمية اقتصادية ملحوظة لأنها تسبب خسائر زراعية كبيرة على الصعيد العالمي. أُجريت هذه الدراسة لتحديد خصائص، وتنوع، وتطور العلاقة، وتصنيف عائلة وعشيرة مورثات البروتين Cyp لأربعة أنواع فطرية تابعة للجنس *Ustilago*، وهي: *U. bullata*، *U. maydis*، *U. hordei* و *U. virens*. وقد استُخدم في هذه الدراسة ما مجموعه 77 مورثاً من مورثات بروتين Cyp لأنواع الفطور آنفة الذكر. تمّ تجميع مورثات البروتين Cyp في خمس مجموعات، وشوهد ما مجموعه 26 عشيرة و 35 عائلة من Cyp؛ وكان توزيع العائلات عبر أنواع الجنس *Ustilago* على الشكل التالي: *U. verens* (20 عائلة)، *U. Maydis* 521 v2 (13 عائلة)، *U. HordeI* Uh 4857 (10 عائلات) و *Bullata* UB2112 (12 عائلة). وكان من المتوقع أن ترتبط عائلات Cyp عبر أنواع الجنس *Ustilago* بالأيض الأولي والثانوي والمواد الغريبة. وقد أشار تحليل الموضعيات الخلوية الفرعية للمورثات CYP450 في أنواع الجنس *Ustilago* إلى أن 88% من البروتينات كانت محصورة في الشبكة البلازمية الداخلية(الإندوبلازمية). وبيّنت الدراسة أن 49 مورثاً فقط من نوع Cyp450 الموجودة في أنواع الفطر *Ustilago* ترتبط بخمس مجموعات جينية ثانوية ذات صلة بالأيض، والتي يمكن تقديرها لأغراض تطبيقات وإدارة هذه العوامل المرضية المهمة في المراحل النهائية. يمكن لهذه الدراسة أن توفر المعرفة الأساسية المطلوبة ذات الصلة بتوصيف فسيولوجيا الكائن، فضلاً عن توفير نظرة متعمقة حول وظائف مورثات (Cyp) وبحوث التنوع لأنواع الجنس *Ustilago*، والتي يمكن توظيفها في تطبيقات عملية قيّمة ولاسيمّا إدارة هذه الممرضات المهمة.

كلمات مفتاحية: CYP450، التنوع، علاقات القرابة، مورثات البروتين، *Ustilago*.

عناوين الباحثين: و.ب. داودا[1*]، إ.ج. إفياني[2]، ب. أبراهام[3]، س.أو. أدتونجي[4]، إ. جلين[5]، إ د. موروميدا[6]، إ.أو. أوجرا[7]، ج.و. بيتر[8]، س.ف. أبراهام[9]، س. شوكوو[3]، ن.ج. داسوم[6]، إم.آي. لوكا[5] وم.ب. أبراهام[3]. (1) قسم الزراعة، جامعة غاشوا الفيدرالية ، ولاية يوبي، نيجيريا؛ (2) قسم علوم المحاصيل، جامعة أويو، أويو PMB 1071، ولاية أكوا إيبوم، نيجيريا؛ (3) قسم تكنولوجيا البستنة، الكلية الفيدرالية للبستنة، PMB 108، دادين كوا، غومبي، نيجيريا؛ (4) مختبر علم الأحياء الدقيقة التطبيقي والتكنولوجيا الحيوية وتكنولوجيا النانو، قسم علم الأحياء الدقيقة، جامعة إيدو إيامهو، PMB 04، أوتشي، ولاية إيدو، نيجيريا؛ (5) قسم الكيمياء الحيوية، جامعة لوكوجا الفيدرالية، Lokoja PMB 1154، ولاية كوجي، نيجيريا؛ (6) قسم علم الأحياء الدقيقة، جامعة ووكاري الفيدرالية، ووكاري PMB 1020، ولاية تارابا، نيجيريا؛ (7) مركز اليونسكو الدولي للتكنولوجيا الحيوية نسوكا، نيجيريا؛ (8) قسم الكيمياء الحيوية، جامعة أحمدو بيلو، زاريا 800001، ولاية كادونا، نيجيريا؛ (9) قسم الزراعة، جامعة بايرو كانو، كانو P.M.B. 3011، ولاية كانو، نيجيريا.
*البريد الإلكتروني للباحث المراسل: wadzanidaudap@fugashua.edu.ng

References

Al-Maaroof, E.M. and P.H. Saed. 2023. Resistance of Iraqi wheat cultivars to common bunt disease and molecular diagnosis of the available *Bt genes* in each cultivar. The Iraqi Journal of Agricultural Sciences, 54:1760-1772. https://doi.org/10.36103/ijas.v54i6.1875

Begerow, D., A.M. Schafer, R. Kellner, A. Yurkov and M. Kemler. 2014. Ustilaginomycotina. Pp. 295-329.

In*:* The mycota volume 7a. Systematics and Evolution, D.J. McLaughlin and J.W. Spatafora (eds.). 2nd edition. Springer, Berlin. https://doi.org/10.1007/978-3-642-55318-9_11

Chadha S., S.T. Mehetre, R. Bansal, A. Kuo, A. Aerts, I.V. Grigoriev, I.S. Druzhinina and P.K. Mukherjee. 2018. Genome-wide analysis of cytochrome P450s of *Trichoderma* spp.: Annotation and evolutionary relationships. Fungal Biology and Biotechnology, 5(1):1-15. https://doi.org/10.1186/s40694-018-0056-3

Cresnar, B. and S. Petric. 2011. Cytochrome P450 enzymes in the fungal kingdom. BiochimBiophys Acta, 1814:29-35. https://doi.org/10.1016/j.bbapap.2010.06.020

Dauda, W.P., P. Abraham, I.V. Fasogbon, C.O. Adetunji, O.O. Banwo, B.D. Kashina and M.D. Alegbejo. 2021a. Cassava mosaic virus in Africa: Functional analysis of virus coat proteins based on evolutionary processes and protein structure. Gene Reports, 24(3):101239. https://doi.org/10.1016/j.genrep.2021.101239

Dauda, W.P., E. Glen, P. Abraham, C.O. Adetunji, D. Morumda, S.E. Abraham, G.P. Wabba, I.O. Ogwuche and M.K. Azameti. 2021b. Comparative Phylogenomic Analysis of Cytochrome P450 Monooxygenases from *Fusarium* species. Research Aquare, 15 pp. https://doi.org/10.21203/rs.3.rs-1097665/v1.

Dauda, W.P., P. Abraham, E. Glen, C.O. Adetunji, S. Ghazanfar, S. Ali, M. Al-Zahrani, M.K. Azameti S.E.L. Alao, A.B. Zarafi, M.P. Abraham and H. Musa. 2022a. Robust Profiling of Cytochrome P450s (P450ome) in Notable *Aspergillus* spp. Life, 12:451. https://doi.org/10.3390/life12030451

Dauda, W.P., D. Morumda, P. Abraham, C.O. Adetunji, S. Ghazanfar, E. Glen, S.E. Abraham, G.P. Wabba, I.O. Ogra, U.I. Ifeanyi, H. Musa, M.K. Azameti, B.A. Paray and A. Gulnaz. 2022b. Genome-Wide Analysis of Cytochrome P450s of *Alternaria* Species: Evolutionary Origin, Family Expansion and Putative Functions. Journal of Fungi 8:324. https://doi.org/10.3390/jof8040324

Dauda, W.P., G.P. Wabba, Abraham P., C.O. Adetunji, E. Glen, M. Daji, I.O. Ogra, E.A. Shittu, M.K. Azameti, S. Ghazanfar, O.O. Osemwegie, O.T. Olaniyan and M.M.C. Anyakudo. 2022c. Bioinformatics Based Structural Analysis of Cytochrome P450 genes in *Candida tropicalis*. Nigerian Journal of Parasitology, 43(2):379-390. https://doi.org/10.4314/njpar.v43i2.17

Dauda, W.P., I.O. Ogra, P. Abraham, C.O. Adetunji, E. Glen, D. Morumda, G.P. Wabba, S.E. Abraham and M.K. Azameti. 2022d. Elucidating the evolutionary and structural features of cytochrome P450 genes in *Cryptococcus neoformans* using in-silico approaches. NABDA Journal of Biotechnology Research, 1(1):5-13.

Dauda, W.P., E. Glen, P. Abraham, C.O. Adetunji, D. Morumda, S.E. Abraham, G.P. Wabba and I.O. Ogwuche. 2023. Elucidating the functional annotation and evolutionary relationships of cytochrome P450 genes in *Xylaria* sp. FL1777 using in-silico approaches. FUDMA Journal of Sciences. 7(4):246-264. https://doi.org/10.33003/fjs-2023-0704-1922

Eccles J.A. and W.S. Baldwin. 2022. Detoxification cytochrome P450s (CYPs) in families 1-3 produce functional oxylipins from polyunsaturated fatty acids. Cells, 12(1):82. https://doi.org/10.3390/cells12010082

Etienne, P.A.N and M. Ingelman-Sundberg. 2008. Intracellular transport and localisation of microsomal cytochrome P450. Analytical and Bioanalytical Chemistry, 392:1075-1084. https://doi.org/10.1007/s00216-008-2200-z

Feyereisen, R. 2011. Arthropod CYPomes illustrate the tempo and mode in P450 evolution. Biochimica et Biophysica Acta, 1814(1):19-28. https://doi.org/10.1016/j.bbapap.2010.06.012

Guengerich, F.P. 2022. Roles of cytochrome P450 enzymes in pharmacology and toxicology: Past, present, and future. Advances in Pharmacology, 95:1-47. https://doi.org/10.1016/bs.apha.2021.12.001

Ichinose, H. 2014. Metabolic diversity and cytochromes P450 of fungi. Pp. 197-205. In: Fifty Years of Cytochrome P450 Research. H.Yamazaki (ed.). Springer, Tokyo, Japan. https://doi.org/10.1007/978-4-431-54992-5_11

Johnston, J.B., H. Ouellet, L.M. Podust and P.R. Ortiz de Montellano. 2011. Structural control of cytochrome P450-catalyzed omega-hydroxylation. Archives in Biochemistry and Biophysics, 507:86-94. https://doi.org/10.1016/j.abb.2010.08.011

Jose, R.C., T. Kanchal, B. Louis, N.C. Talukdar and D. Chowdhury. 2023. Grain characteristics, moisture, and specific peptides produced by ustilaginoidea virens contribute to false smut disease in rice (*Oryza sativa* L.). Biomolecules, 13(4):669. https://doi.org/10.3390/biom13040669

Kelly, S.L. and D.E. Kelly. 2013. Microbial cytochromes P450: biodiversity and biotechnology. Where do cytochromes P450 come from, what do they do and what can they do for us? Philosophical Transactions of the Royal Society B Biological Sciences, 368(1612):20120476. https://doi.org/10.1098/rstb.2012.0476

Kelly, D.E., N. Kraševec, J. Mullins and D.R. Nelson. 2009. The CYPome (cytochrome P450 complement) of *Aspergillus nidulans.* Fungal Genetics and Biology, 46(1):S53-S61. https://doi.org/10.1016/j.fgb.2008.08.010

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6):1547-1549. https://doi.org/10.1093/molbev/msy096

Lah, L., S. Haridas, J. Bohlmann and C. Breuil. 2013. The cytochromes P450 of Grosmanniaclavigera: genome organisation, phylogeny, and expression in response to pine host chemicals. Fungal Genetics and Biology, 50:72-81. https://doi.org/10.1016/j.fgb.2012.10.002

Lepesheva, G.I. and M.R. Waterman. 2007. Sterol 14alpha-demethylase cytochrome P450 (CYP51), a

P450 in all biological kingdoms. Biochimica et biophysica acta (BBA)-General subjects, 1770(3):467-477. https://doi.org/10.1016/j.bbagen.2006.07.018

Lepesheva, G.I. and M.R. Waterman. 2011. Structural basis for conservation in the CYP51 family. Biochimica et biophysica acta (BBA)-General subjects, 1814:88-93. https://doi.org/10.1016/j.bbapap.2010.06.006

Liu, J., H. Hu, H. Shen, Q. Tian, W. Ding, X. Yang, L. Wang, and Y. Yue. 2022. Insights into the cytochrome P450 monooxygenase superfamily in Osmanthus fragrans and the role of CYP142 in linalool synthesis. International Journal of Molecular Sciences, 23(20):12150. https://doi.org/10.3390/ijms232012150

McTaggart, A.R., R.G. Shivas, A.D.W. Geering, B. Callaghan, K. Vánky and T. Scharaschkin. 2012. Soral synapomorphiles are siginificant for the systematics of the *Ustilago sporisorium macalpinomyces* complex (Ustilaginaceae). Persoonia, 29:63-77. https://doi.org/10.3767/003158512X660562

Nelson D.R, M.A. Schuler, S.M. Paquette, D. Werck-Reichhart and S. Bak. 2004. Comparative genomics of rice and Arabidopsis. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. Plant Physiology, 135(2):756-772. https://doi.org/10.1104/pp.104.039826

Nelson, D.R. 1999. Cytochrome P450 and the individuality of species. Archives of biochemistry and biophysics, 369(1):1-10.

Nelson, D.R. 2006. Cytochrome P450 Nomenclature. Methods in Molecular Biology, 320:1-10.

Newsome, A.W., D. Nelson, A. Corran, S.L. Kelly and D.E. Kelly. 2013. The cytochrome P450 complement (CYPome) of *Mycosphaerella graminicola.* Biotechnology and Appllied Biochemistry, 60:52-64. https://doi.org/10.1002/bab.1062

Oekmen, B., D. Schwammbach, G. Bakkeren, U. Neumann and G. Doehlemann. 2021. The *Ustilago hordei* barley interaction is a versatile system for characterization of fungal effectors. Journal of Fungi, 7(2):86. https://doi.org/10.3390/jof7020086

Ruiz-Herrera, J. and C.G. León-Ramírez. 2012. Development and dimorphism of the pathogenic basidiomycota *Ustilago maydis*. Pp. 105-116. In*:* Dimorphic Fungi: Their Importance as Models for Differentiation and Pathogenesis. J. Ruiz-Herrera (ed.). Bentham, UK.

Saha, P., A. Sarkar, N. Sabnam, M.D. Shirke, H.B. Mahesh, A. Nikhil, A. Rajamani, M. Gowda and S. Roy-Barman. 2021. Comparative analysis of secondary metabolite gene clusters in different strains of *Magnaporthe oryzae*. FEMS Microbiology Letters, 368(1):fnaa216. https://doi.org/10.1093/femsle/fnaa216

Sello, M.M., N. Jafta, D.R. Nelson, W. Chen, J.H. Yu, M. Parvez, I.K.R. Kgosiemang, R. Monyaki, S.C. Raselemane, L.B. Qhanya, N.T. Mthakathi, S. Sitheni Mashele and K. Syed. 2015. Diversity and evolution of cytochrome P450 monooxygenases in Oomycetes. Scientific Reports, 5:11572. https://doi.org/10.1038/srep11572

Singh, A.K. and D.J. Pophaly. 2010. An unusual rice false smut epidemic reported in Raigarh District, Chhattisgarh. International Rice Research Notes, 35:1-3.

Syed, K. and J.S. Yadav. 2012. P450 monooxygenases (P450ome) of the model white rot fungus *Phanerochaete chrysosporium.* Critical Reviews in Microbiology, 38(4):339-63. https://doi.org/10.3109/1040841X.2012.682050

Syed, K. and S.S. Mashele. 2014. Comparative analysis of P450 signature motifs EXXR and CXG in the large and diverse kingdom of fungi: identification of evolutionarily conserved amino acid patterns characteristic of P450 family. PloSone, 9(4):e95616. https://doi.org/10.1371/journal.pone.0095616

Ullmann, L., D. Wibberg, T. Busche, C. Rückert, A. Müsgens, J. Kalinowski and L.M. Blank. 2022. Seventeen Ustilaginaceae high-quality genome sequences allow phylogenomic analysis and provide insights into secondary metabolite synthesis. Journal of Fungi, 8(3):269. https://doi.org/10.3390/jof8030269

Wang, Z., A. Romanski, V. Mehra, Y. Wang, B.C. Campbell, G.A. Petsko, P. Tsoulfas and M. Blackmore. 2021. Brain-wide analysis of the supraspinal connectome reveals anatomical correlates to functional recovery after spinal injury. bioRxiv, 2021:447885. https://doi.org/10.1101/2021.06.10.447885

Werck-Reichhart, D. and R. Feyereisen. 2000. Cytochromes P450: A success story. Genome Biology, 1(6):1-9

Wu, Y., M. Wu, Y. Wang, Y. Chen, J. Gao and C. Ying. 2018. ERG11 couples oxidative stress adaptation, hyphal elongation, and virulence in *Candida albicans*. FEMS Yeast Research, 18:foy057. https://doi.org/10.1093/femsyr/foy057

Zarafi, A.B. and W.P. Dauda. 2019. Exploring the importance of fungi in agricultural biotechnology. International Journal of Agricultural Science and Veterinary Medicine, 7(1):1-12

Zhang, J., L. Li, Q. Lv, L. Yan, Y. Wang and Y. Jiang. 2019. The Fungal CYP51s: Their Functions, structures, related drug resistance, and inhibitors. Frontiers in Microbiology, 10:691. https://doi.org/10.3389/fmicb.2019.00691

Zuo, W., B. Ökmen, J.R. Depotter, K.M. Ebert, A. Redkar, M. J. Villamil and G. Doehlemann. 2019. Molecular interactions between smut fungi and their host plants. Annual Review of Phytopathology, 57:411-430. https://doi.org/10.1146/annurev-phyto-082718-100139

Received: September 17, 2023; Accepted: February 27, 2024

تاريخ الاستلام: 2023/9/17؛ تاريخ الموافقة على النشر: 2024/2/27