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Mechanism of Resistance TAF(II) Protein of *Aspergillus salvadorensis* to Oxidative Stress in its Environment

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Abstract:

In this study, bioinformatics analysis of the protein sequences of the *Aspergillus salvadorensis* genome was carried out, with the aim of identifying key proteins that participate in metabolic, structural and regulatory processes. Through functional annotation tools and comparison with protein databases, several proteins with relevant biological functions were found, including ferrochelatase, the mitochondrial transporter of thiamine pyrophosphate, annexins and hydrolytic enzymes such as endo-β-1,4-glucanase. These proteins play critical roles, such as heme biosynthesis, mitochondrial energy metabolism, cellular homeostasis, and remodeling of the fungal cell wall. Comparative sequence analysis showed that many of these proteins are very similar to those of other filamentous fungi, especially *Neurospora crassa*, indicating a strong evolutionary conservation of genes involved in essential metabolic pathways. In addition, proteins related to the regulation of gene transcription were identified, such as the TAF(II) subunit of the TFIID complex, which helps initiate RNA polymerase II-dependent transcription and regulates gene expression in the face of changes in the environment. This protein allows *A. salvadorensis* to precisely control the production of industrial enzymes and secondary metabolites, ensuring that they are generated at the right time and place within the cell. The hydrolytic enzymes detected also suggest that the fungus has a strong ability to remodel its cell wall and possibly to degrade complex polymers present in its environment. This characteristic is common in filamentous fungi that live in environments rich in organic matter, where biomass degradation is key to obtaining nutrients. The results show that *A. salvadorensis* has a complex metabolic and regulatory network, which allows it to adapt to the environment, grow efficiently and resist certain antifungal compounds. Likewise, its repertoire of enzymes and specialized metabolic systems highlights its potential biotechnological value, with applications in biodegradation, production of industrial enzymes and studies of molecular biology of fungi.

Keywords: *Aspergillus salvadorensis*, TAF protein, ferrochelatase, endo-β-1,4-glucanase.

INTRODUCTION

The genus Aspergillus comprises a diverse group of filamentous fungi that are widely distributed in different terrestrial ecosystems, including soils, decomposing organic waste, agricultural environments, and industrial systems (Brakhage, 2013; Echevarría, 2019; Echevarría and Iqbal, 2021; Iqbal *et al.*, 2019). These species stand out for their remarkable metabolic versatility, which allows them to degrade a wide variety of complex organic compounds and adapt to changing environmental conditions. This capacity for adaptation and functional diversity has made members of the genus Aspergillus important study models for biotechnology, environmental microbiology and molecular genetics, as well as potential sources of industrial enzymes and secondary metabolites of pharmacological and biotechnological interest (Brakhage, 2013; Krijgsheld *et al.*, 2013).

In recent years, advances in genomic sequencing technologies have opened up new opportunities to explore the genetic and functional diversity of filamentous fungi in greater detail. Whole-genome analysis has revealed that many *Aspergillus* species possess a broad repertoire of genes encoding extracellular enzymes, regulatory proteins, and specialized metabolic systems. These molecules allow fungi to degrade natural polymers such as cellulose, hemicellulose, chitin, and other complex components of organic matter. The enzymes involved in these processes, particularly hydrolases, play a central role in the biogeochemical cycles of carbon and nitrogen, facilitating the decomposition of plant biomass and other organic materials in terrestrial ecosystems (Gavande and Goyal, 2023; Imran *et al.*, 2019; Kulshreshtha and Sharma, 2022).

The study of new species or strains of Aspergillus is especially relevant to understand the molecular mechanisms that support its metabolism and adaptive capacity. One of these species is *Aspergillus salvadorensis*, a filamentous fungus whose genomic characterization is beginning to reveal a diverse repertoire of proteins involved in essential metabolic processes (Vásquez, 2025). Among the proteins of interest identified in genomic studies is ferrochelatase, an enzyme responsible for catalyzing the final step in the biosynthesis of the heme group by inserting a ferrous ion (Fe^{2+}) into protoporphyrin IX. Heme is a fundamental cofactor in numerous proteins involved in electron transport and redox reactions, including cytochromes and other enzymes of the mitochondrial respiratory chain. The presence of ferrochelatase suggests that *A. salvadorensis* has an active iron metabolism and aerobic respiration system, essential elements for cellular energy production and the maintenance of energy metabolism (Dailey *et al.*, 2017; Sil and Chakraborti, 2025).

Another relevant protein identified corresponds to the thiamine pyrophosphate mitochondrial transporter, known as Thiamine Pyrophosphate Carrier 1. This transporter mobilizes thiamine pyrophosphate into the mitochondrial matrix, where it acts as a cofactor in critical metabolic reactions, particularly in the decarboxylation of α-ketoacids. These reactions are key components of fundamental metabolic pathways, such as the tricarboxylic acid cycle and carbohydrate metabolism, essential for energy generation and cellular homeostasis (Marobbio *et al.*, 2002).

In addition to proteins involved in metabolism, proteomic analysis of *A. salvadorensis* has identified structural and regulatory proteins, such as annexins. This family of proteins interacts with phospholipid membranes in the presence of calcium and is involved in essential cellular processes such as intracellular signaling, exocytosis, and membrane repair. In filamentous fungi, annexins contribute to maintaining calcium homeostasis and the integrity of cell membranes, facilitating adaptation to environmental changes and survival under fluctuating conditions (Gerke and Moss, 2002).

Among the hydrolytic enzymes detected, endo-β-1,4-glucanase stands out, which participates in the degradation of structural polysaccharides by breaking β-1,4-glycosidic bonds present in glucans. This type of enzyme is not only

essential for cell wall remodeling during growth and differentiation processes, but also allows fungi to degrade complex polymers from the environment, releasing simpler molecules that can be used as sources of carbon and energy (Gavande and Goyal, 2023). In addition to metabolic and structural proteins, genomic analysis of *Aspergillus salvadorensis* has identified proteins involved in the regulation of gene expression. These include the TAF(II) subunit, which is part of the TFIID complex, an essential component of the eukaryotic transcription machinery. This complex recognizes promoter sequences in DNA, such as the TATA box, and coordinates the assembly of RNA polymerase II along with other transcription factors needed to initiate messenger RNA synthesis. Beyond their structural role, TAF proteins are involved in interacting with transcriptional activators and chromatin remodeling, thereby modulating DNA accessibility and regulating the activity of the transcription machinery (Nogales *et al.*, 2017; Thomas and Chiang, 2006).

In fungi of the genus Aspergillus, transcriptional regulation is crucial to control key physiological processes, such as mycelial growth, spore formation, and the production of secondary metabolites. These metabolites include bioactive compounds of high biotechnological interest, such as industrial enzymes, antibiotics and mycotoxins. The synthesis of these compounds is generally organized into specific gene clusters, whose activation depends on complex transcriptional regulation systems (Brakhage, 2013).

Likewise, several studies have shown that gene regulation mechanisms in fungi play a central role in the response to environmental stress conditions, such as oxidative stress, nutrient limitation or exposure to antifungal compounds. Regulatory systems allow the body to quickly adjust its metabolism, maintaining cell viability and growth capacity in the face of adverse conditions (Corrêa-Junior *et al.*, 2026; Cowen *et al.*, 2015).

The genomic and proteomic analysis of *A. salvadorensis* provides an initial insight into the complex metabolic and regulatory network that underpins the physiology of this fungus. The identification of metabolic enzymes, structural proteins, and transcriptional regulation factors suggests that the species has sophisticated mechanisms to adapt to different environments and take advantage of diverse sources of nutrients. In addition, the presence of hydrolytic enzymes and regulatory proteins reinforces the biotechnological potential of *A. salvadorensis*, especially in applications related to biomass degradation, the production of industrial enzymes and the study of the molecular biology of filamentous fungi.

MATERIALS AND METHODS

For this study, the DNA sequence obtained from *Aspergillus salvadorensis*, provided by MACROGEN SOUTH KOREA (2025), was used. This genomic sequence was organized in FASTA format, which facilitated its analysis using various bioinformatics tools, allowing the data to be prepared for the identification of coding regions, the prediction of potential proteins and the exploration of their biological functions within the metabolism of the fungus. Prominent proteins include ferrochelatase (UniRef90_A2R528), involved in the production of the heme group, and the TAF(II) subunit (UniRef90_A2R5B6), an essential component of the TFIID complex for the regulation of gene transcription.

The analysis began with the identification of open reading frames (ORF) using the National Center for Biotechnology Information's (NCBI) ORF Finder tool (Wheeler *et al.*, 2008). This tool allows detecting potentially coding regions by recognizing start codons, mainly ATG, and end codons, such as TAA, TAG or TGA. The search was carried out considering the six possible reading patterns of the sequence, three in the direct strand and three in the complementary strand. The identified ORFs were evaluated based on their length and biological coherence,

selecting those with the highest probability of encoding functional proteins (Lei *et al.*, 2022).

Subsequently, the predicted protein sequences were compared with international databases using BLASTp, also available in NCBI (Bharose *et al.*, 2024). This analysis allowed the identification of homologous proteins present in other organisms and the degree of similarity between the sequences obtained and previously characterized proteins. To interpret the results, parameters such as the percentage of identity, the coverage of the alignment and the E-value, which indicates the probability that a match occurs by chance, were considered. Low E-value values suggest significant evolutionary relationships between the proteins compared.

To assign possible biological functions to the identified proteins, a functional annotation was made by comparison with the UniProt and UniRef90 databases (Talamantes-Becerra *et al.*, 2026). These platforms contain extensive collections of protein sequences organized according to structural and functional similarity, which allows predicting biological functions from conserved domains, even in genes that have not yet been experimentally characterized. This approach is widely used in genomic studies to infer the metabolic and physiological role of novel proteins.

In addition, the evolutionary relationship of proteins was explored through comparative phylogenetic analyses with homologous sequences of related species of the genus *Aspergillus*. The protein sequences were aligned using multiple alignment methods, allowing the identification of conserved regions and the evaluation of evolutionary divergence. Based on these alignments, phylogenetic trees were constructed that provided information on the evolutionary relationships between the proteins studied and their counterparts in other filamentous fungi (Zhang *et al.*, 2024).

Finally, the results obtained from all the bioinformatics tools were integrated and analyzed jointly. This approach allowed us to identify key proteins related to metabolic, structural and regulatory processes in *A. salvadorensis*, and to generate inferences about their possible role in the physiology of the organism. Highlighted functions include adaptation to different environments, efficient energy metabolism and the production of enzymes with biotechnological potential.

Overall, bioinformatics analysis provides a solid foundation for future experimental research aimed at understanding the molecular biology of *A. salvadorensis*. In addition, it highlights the potential of this species for applications in biotechnology, such as the production of industrial enzymes, and in bioremediation, through the degradation of biomass and other complex organic compounds present in its environment.

RESULTS

The functional annotation analysis of the genome of *Aspergillus salvadorensis*, carried out using orthology tools and databases such as UniRef, made it possible to identify proteins with conserved functions in other eukaryotic organisms, especially in filamentous fungi (Table 1). Among these annotations, an orthologous protein linked to the TFIID transcription initiation complex stands out, with functional similarity to the TAF47 subunit (Transcription initiation factor TFIID subunit 47) described in the model yeast *Saccharomyces cerevisiae*. Proteins of the TAF family (TBP-associated factors) are part of the TFIID multiprotein complex, which plays a crucial role in the recognition of gene promoters and in the recruitment of RNA polymerase II during the initiation of transcription. The presence of this protein in *A. salvadorensis* suggests that the fundamental regulatory mechanisms for gene expression are conserved in filamentous fungi, reflecting a molecular organization similar to that observed in other members of the genus *Aspergillus*.

In addition, the bioinformatics analysis made it possible to identify proteins classified as hydrolases, associated with the

UniRef90_D9X6H6 protein cluster. Hydrolases constitute one of the main enzymatic classes in saprophytic fungi, as they catalyze hydrolysis reactions that break chemical bonds by incorporating water molecules. In species of the genus *Aspergillus*, these enzymes play an active role in the breakdown of naturally occurring polysaccharides, proteins, lipids, and other polymers present in the environment. Therefore, the identification of genes encoding hydrolases in *A. salvadorensis* supports their metabolic potential to exploit complex substrates and underscores their potential ecological contribution to the degradation and recycling of organic matter in tropical ecosystems.

Table 1. Result of the sequencing of Aspergillus salvadorensis 2024. MACROGEN INC.

COG ID Aspergillus	Orthology	MetaCyc/EggNOG/KEGG/Uniref90/K/KEGGsummary
UniRef90_A2R5B6 26542	Remark_TAF47 S. cerevisiae is a TAF(II) complex	35.863
UniRef90_D9X6H6 27857	Hydrolase	0.532662
UniRef90_A2R4W4 26512	Remark_the main function of the ATPases is the adaptation to stress conditions	30.8744
UniRef90_A2R528	Ferroquetalasa	0.0961474
ENOG410XP44 949	Alpha beta hydrolase fold protein	1.17334
ENOG410Y3MI 985	hydrolase family 38	0.43406
ENOG4111GJ1 1024	Alpha beta hydrolase	0.532282

Figure (1) presents a genetic sequence in FASTA format belonging to a fungus of the genus *Aspergillus*. The file name and sequence header indicate that it is *Aspergillus salvadorensis* 23.2 kb, a species whose name suggests a possible link with taxonomic studies carried out in the region of El Salvador. This type of sequence is commonly used as a "molecular barcode", a tool widely used in mycology to identify species with similar morphological characteristics, which are difficult to differentiate by traditional methods.

It is likely that the sequence corresponds to one of the most widely used molecular markers for the identification of fungi. These include the Internal Transcribed Spacer (ITS), considered the leading standard in molecular identification studies, and the calmodulin gene, which offers higher resolution to differentiate closely related species within the genus *Aspergillus*.

The length of the sequence suggests that it is a relatively broad fragment, which could range from hundreds to several thousand base pairs. This size is consistent with genomic regions used in molecular identification studies, such as ITS spacers, ribosomal DNA segments, or conserved metabolic genes. These regions present a combination of highly conserved areas, which allow alignment between species, and more variable regions, which provide phylogenetic information to distinguish closely related organisms.

In terms of its composition, the sequence shows a balanced distribution of nucleotides, a common feature in many fungal genomes. Some regions have higher guanine and cytosine (GC) content, which may indicate structurally more stable segments. GC pairs form three hydrogen bonds, which confer greater stability to the double helix and are usually associated with coding zones or functionally important ribosomal structures.

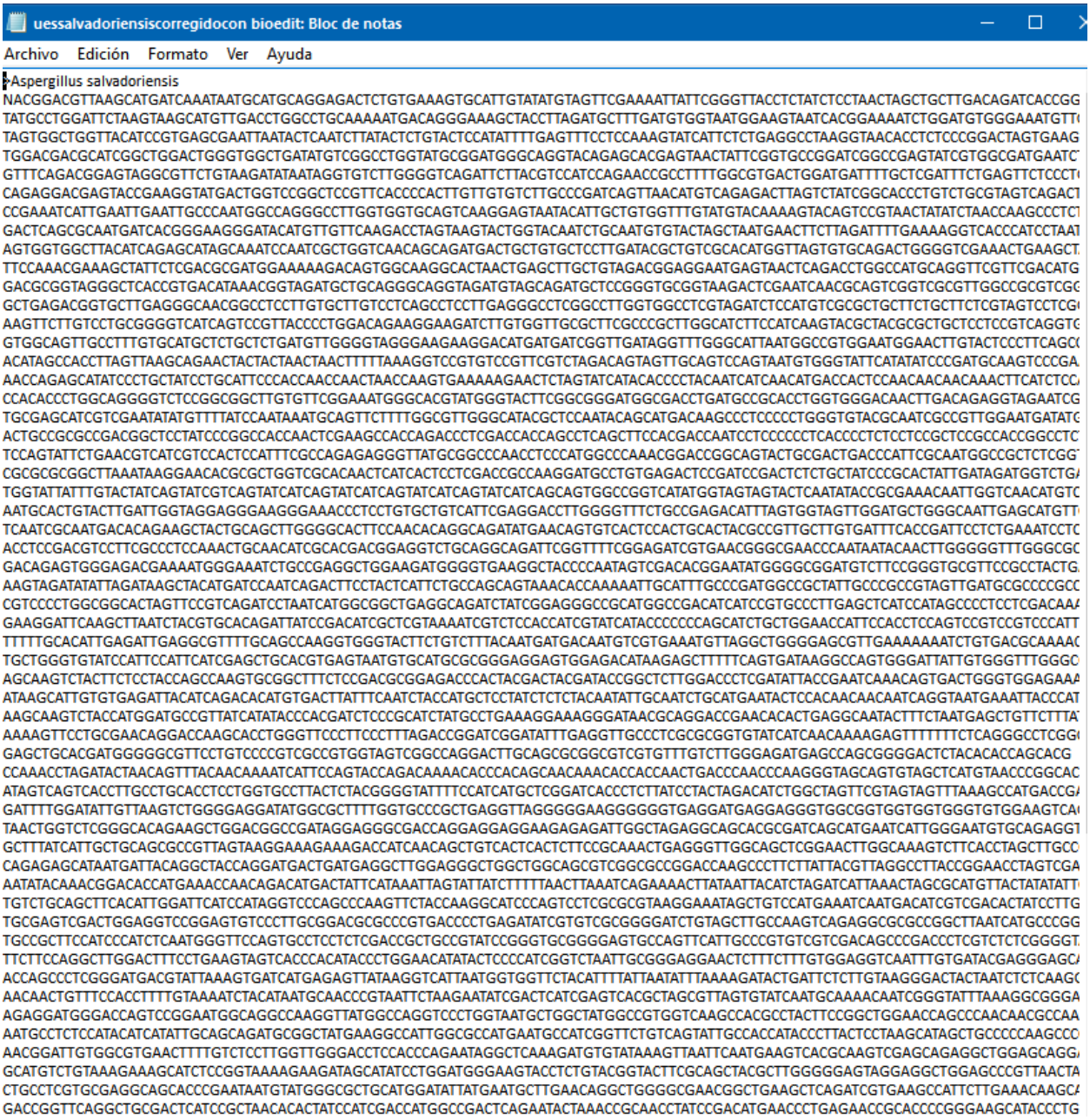


Fig. 1. Species-specific DNA sequence in FASTA format for *Aspergillus salvadorensis*. Macrogen inc. 2026

The analyzed file contains a DNA sequence composed of the bases adenine (A), thymine (T), guanine (G), and cytosine (C). The presence of a header preceded by the symbol ">" followed by the nucleotide chain is characteristic of the FASTA format, widely used in genomic databases and in bioinformatics analysis programs such as BLAST, BioEdit and MEGA. This format allows biological sequences to be stored and facilitates their comparison with other sequences available in international databases.

The overall pattern of the sequence combines conserved segments with variable regions, an organization typical of molecular markers used in fungal taxonomy, particularly ITS. Conserved regions facilitate alignment between distinct organisms, while variable zones allow species within the same genus to be differentiated.

From a functional perspective, if the fragment analyzed corresponds to a coding gene, it is

possible to identify open reading frames (ORF) that could be translated into proteins involved in cell metabolism, the response to environmental stress or the maintenance of cell structure. On the other hand, if the sequence belongs to ribosomal DNA, its main function would be related to the production of ribosomal RNA and the formation of ribosomes, essential for protein synthesis.

In evolutionary terms, this sequence can be used to perform comparative phylogenetic analyses. Through bioinformatic alignment tools, such as BLAST or phylogenetic analysis programs, it is possible to compare the sequence with other homologues of related Aspergillus species. The nucleotide differences detected, such as point mutations, insertions or deletions, allow inferring evolutionary relationships and constructing phylogenetic trees that reflect the divergence between species.

Another important use of this type of sequence is the design of primers for PCR. Certain regions of DNA, with a balanced composition of nucleotides and without extensive repeats, are ideal for designing specific primers. These allow DNA fragments to be amplified in subsequent molecular identification or genetic characterization studies.

Beyond its taxonomic utility, the sequence analyzed offers bioinformatic information on possible metabolic functions. Some fragments could correspond to genes that encode hydrolytic enzymes, such as cutinases, involved in the degradation of cutin present in the cuticle of plants. In Aspergillus, these enzymes are key to obtaining nutrients from organic materials and may have ecological relevance, as they participate in the decomposition of biomass, as well as biotechnological potential in the production of industrial enzymes.

The bioinformatic analysis of the sequences associated with the genome of *Aspergillus salvadorensis* allowed the identification of a diverse set of proteins involved in fundamental metabolic, structural and regulatory processes. Among the proteins highlighted are enzymes involved in the biosynthesis of essential cofactors, mitochondrial transporters, hydrolases and transcription regulatory factors.

Among the metabolic proteins, ferrochelatase was identified, responsible for the final step in the biosynthesis of the heme group. This enzyme catalyzes the insertion of a ferrous ion into protoporphyrin IX, generating the heme group that acts as a cofactor in various respiratory proteins, including cytochromes. The presence of this enzyme indicates an active metabolic system related to cellular respiration and iron metabolism.

Likewise, a mitochondrial thiamine pyrophosphate transporter was detected, responsible for mobilizing this molecule towards the mitochondrial matrix, where it acts as a cofactor in key metabolic reactions, especially in the decarboxylation of α-ketoacids that participate in the Krebs cycle. The identification of this transporter suggests that *A. salvadorensis* has an efficient energy metabolism system.

Among the structural proteins, conserved domains associated with Annexin-type proteins were found, which participate in the interaction with cell membranes and in calcium-dependent signaling processes. In filamentous fungi, these proteins contribute to the organization of membranes during hyphae growth and to the response to environmental stress conditions.

The functional analysis also revealed the possible presence of hydrolases, such as endo-β-1,4-glucanase, involved in the degradation of polysaccharides such as cellulose. These enzymes are critical for cell wall remodeling during spore growth and germination, as well as for the decomposition of plant organic matter in the environment. The presence of these proteins highlights both the ecological importance of *A. salvadorensis* in nutrient recycling processes and its potential application in biotechnology.

The quantitative parameters associated with these proteins, such as 29.4366, 31.4809 and 31.2345, probably correspond to values derived from sequence similarity analyses or alignment

scores in protein databases, reflecting the degree of agreement with previously characterized proteins.

The comparative sequence analysis showed a high similarity between the proteins of *A. salvadorensis* and those described in other filamentous fungi, particularly in Neurospora crassa, which evidences the evolutionary conservation of genes involved in essential cellular functions. In addition, some sequences showed similarities with proteins from phylogenetically more distant organisms, indicating the conservation of fundamental structural domains in eukaryotic proteins.

Additionally, proteins related to antifungal resistance mechanisms were identified, including possible associations with resistance to fluconazole, a compound that inhibits enzymes involved in the biosynthesis of ergosterol, a key component of the fungal cell membrane. The presence of these proteins suggests that *A. salvadorensis* could have cellular systems capable of modulating its sensitivity to antifungal compounds, providing information on its adaptive potential in chemically stressed environments.

These results indicate that the genomic fragment studied contains both ribosomal regions, useful for molecular identification, and coding segments associated with metabolic, structural and regulatory functions. This organization is characteristic of filamentous fungi with high metabolic plasticity and ability to adapt to variable environmental conditions. The findings provide a valuable basis for future experimental studies aimed at exploring the molecular biology, metabolic physiology and biotechnological potential of *Aspergillus salvadorensis*, including applications in biomass degradation, industrial enzyme production and genetic diversity analysis.

The proteomic analysis allowed the identification of regulatory proteins, among which TAF(II) stands out, an integral factor of the TFIID complex involved in the regulation of gene transcription. This complex plays a fundamental role in the recognition of promoter sequences and in the activation of RNA polymerase II, facilitating the initiation of transcription in eukaryotic cells. The presence of TAF(II) in *Aspergillus salvadorensis* suggests the conservation of essential regulatory mechanisms for gene expression, similar to those observed in other filamentous fungi and model eukaryotes.

The characterization of proteins in databases is based on standardized data structures that allow their identification and functional contextualization. Each protein entry is defined by a Unique Identifier, such as UniRef90_A2R528, which acts as a primary and canonical reference within the repository. This system facilitates the comparison, annotation and study of homologous proteins, as well as the prediction of biological functions based on conserved domains and evolutionary similarities.

The above sequence is passed to:

Analysis of open reading frames (ORF) using the National Center for Biotechnology Information (NCBI) ORF viewer revealed the presence of multiple potentially coding regions distributed along the genomic sequence of *Aspergillus salvadorensis* (Figure 2). These regions correspond to segments of DNA that could be translated into functional proteins within the fungal genome. In the visualization, ORF was observed in different reading frames, both in the direct and complementary chains, a common feature in fungal genomes, where genes or gene fragments can be close or overlapping.

Among the identified ORFs, some with significant lengths stand out, such as ORF82, ORF30 and ORF75, which exceed 300 nucleotides, which increases the probability that they correspond to functional genes. These regions were selected for further analysis using sequence comparison tools such as BLAST, with the aim of identifying similarities with proteins previously characterized in biological databases.

The alignment results suggest that several of these coding regions are similar to proteins of

the fumarylacetoacetate hydrolase family, a group of enzymes classified within the hydrolases. These enzymes participate in metabolic processes of degradation of organic compounds, which indicates that the analyzed strain has metabolic capacities related to the transformation and recycling of metabolites in its environment, a common characteristic in saprophytic fungi.

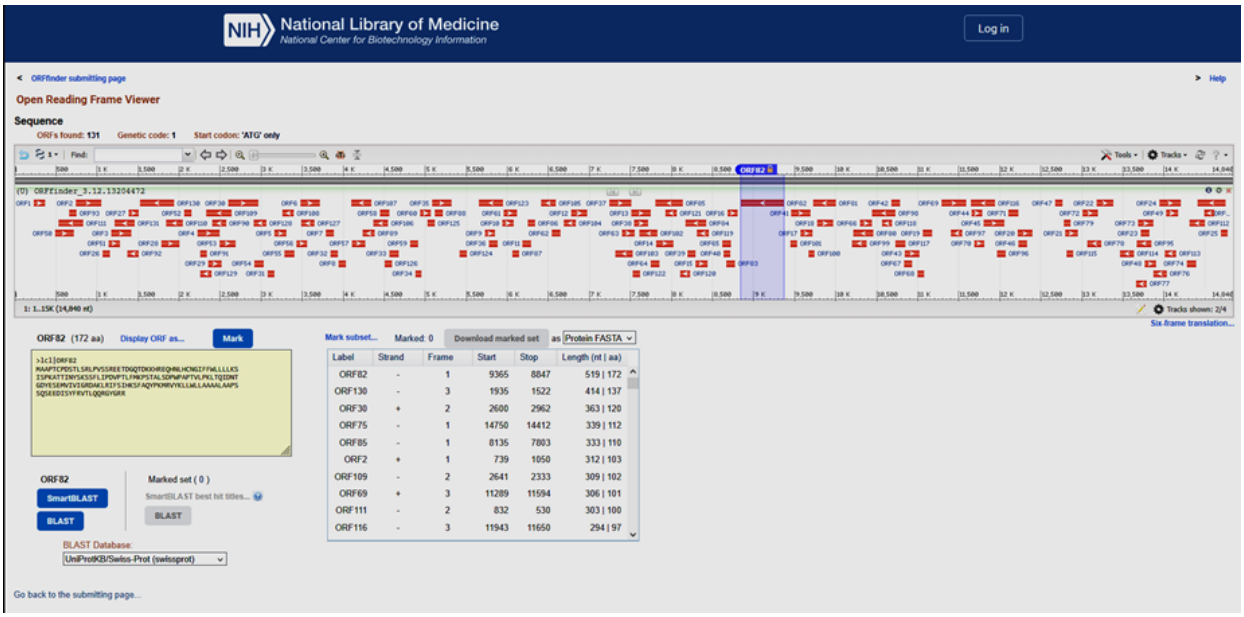


Fig. 2. Open ORF reading frame of the sequence *A. salvadorensis*. ORFinder 2025

However, from the analysis of the ORFs and the alignment results, no sequences were identified that specifically correspond to ferrochelatase, an enzyme involved in the biosynthesis of the heme group, or to the mitochondrial thiamine pyrophosphate transporter, responsible for the transport of vitamin B1-derived cofactors to the mitochondria. Similarly, proteins of the annexin family, associated with membrane organization and cell signaling, were not detected, nor were specialized hydrolytic enzymes such as endo-β-1,4-glucanase, involved in cellulose degradation.

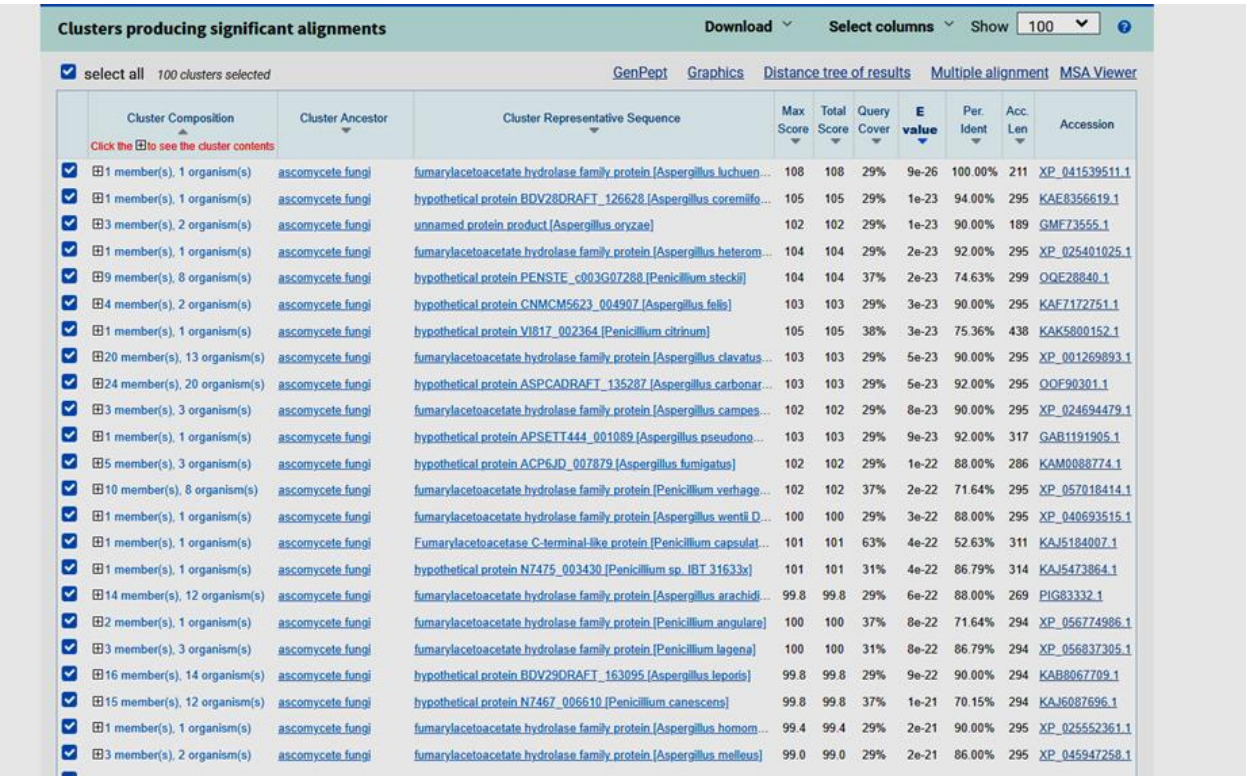
The sequence studied in *A. salvadorensis* mainly encodes proteins related to metabolic hydrolases conserved in ascomycete fungi. This information provides valuable data on the genetic organization and metabolic potential of the species within its ecological niche. In addition, it constitutes a first step for the functional characterization of the genome and serves as a basis for future genomic annotation and experimental validation studies.

This ORF 82 protein is passed to BLASTp and remains:

The analysis of the alignments obtained indicates that the sequence studied in *Aspergillus salvadorensis* shows a significant similarity with proteins of the fumarylacetoacetate hydrolase family, a group of enzymes classified within the hydrolases (Figure 3). These proteins catalyze hydrolysis reactions involved in metabolic pathways related to the degradation of organic compounds and the metabolism of aromatic amino acids. The recurrence of alignments with homologous proteins of various species of *Aspergillus and Penicillium*, accompanied by high identity values

and very low E values, suggests that the sequence probably corresponds to a gene conserved within the fungi of the phylum Ascomycota, supporting its metabolic role in enzymatic degradation processes.

However, the results do not show annotations corresponding to enzymes such as ferrochelatase, key in the biosynthesis of the heme group, or to the mitochondrial thiamine pyrophosphate transporter, involved in the transport of vitamin B1-derived cofactors to the mitochondria. Similarly, no proteins of the annexin family, which participate in cell signaling and membrane organization, were identified, nor specialized hydrolases such as endo-β-1,4-glucanase, which is involved in the degradation of structural polysaccharides such as cellulose.



Clusters producing significant alignments Download Select columns Show 100

select all *100 clusters selected* GenPept Graphics Distance tree of results Multiple alignment MSA Viewer

Cluster Composition (Click the ⊞ to see the cluster contents)	Cluster Ancestor	Cluster Representative Sequence	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
⊞1 member(s), 1 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus luchuen...	108	108	29%	9e-26	100.00%	211	XP_041539511.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	hypothetical protein BDV28DRAFT_126628 [Aspergillus coremiifo...	105	105	29%	1e-23	94.00%	295	KAE8356619.1
⊞3 member(s), 2 organism(s)	ascomycete fungi	unnamed protein product [Aspergillus oryzae]	102	102	29%	1e-23	90.00%	189	GMF73555.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus heterom...	104	104	29%	2e-23	92.00%	295	XP_025401025.1
⊞9 member(s), 8 organism(s)	ascomycete fungi	hypothetical protein PENSTE_c003G07288 [Penicillium steckii]	104	104	37%	2e-23	74.63%	299	OQE28840.1
⊞4 member(s), 2 organism(s)	ascomycete fungi	hypothetical protein CNMCM5623_004907 [Aspergillus felis]	103	103	29%	3e-23	90.00%	295	KAF7172751.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	hypothetical protein VI817_002364 [Penicillium citrinum]	105	105	38%	3e-23	75.36%	438	KAK5800152.1
⊞20 member(s), 13 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus clavatus...	103	103	29%	5e-23	90.00%	295	XP_001269893.1
⊞24 member(s), 20 organism(s)	ascomycete fungi	hypothetical protein ASPCADRAFT_135287 [Aspergillus carbonar...	103	103	29%	5e-23	92.00%	295	OOF90301.1
⊞3 member(s), 3 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus campes...	102	102	29%	8e-23	90.00%	295	XP_024694479.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	hypothetical protein APSETT444_001089 [Aspergillus pseudono...	103	103	29%	9e-23	92.00%	317	GAB1191905.1
⊞5 member(s), 3 organism(s)	ascomycete fungi	hypothetical protein ACP6JD_007879 [Aspergillus fumigatus]	102	102	29%	1e-22	88.00%	286	KAM0088774.1
⊞10 member(s), 8 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Penicillium verhage...	102	102	37%	2e-22	71.64%	295	XP_057018414.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus wentii D...	100	100	29%	3e-22	88.00%	295	XP_040693515.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	Fumarylacetoacetase C-terminal-like protein [Penicillium capsulat...	101	101	63%	4e-22	52.63%	311	KAJ5184007.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	hypothetical protein N7475_003430 [Penicillium sp. IBT 31633x]	101	101	31%	4e-22	86.79%	314	KAJ5473864.1
⊞14 member(s), 12 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus arachidi...	99.8	99.8	29%	6e-22	88.00%	269	PIG83332.1
⊞2 member(s), 1 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Penicillium angulare]	100	100	37%	8e-22	71.64%	294	XP_056774986.1
⊞3 member(s), 3 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Penicillium lagena]	100	100	31%	8e-22	86.79%	294	XP_056837305.1
⊞16 member(s), 14 organism(s)	ascomycete fungi	hypothetical protein BDV29DRAFT_163095 [Aspergillus leporis]	99.8	99.8	29%	9e-22	90.00%	294	KAB8067709.1
⊞15 member(s), 12 organism(s)	ascomycete fungi	hypothetical protein N7467_006610 [Penicillium canescens]	99.8	99.8	37%	1e-21	70.15%	294	KAJ6087696.1
⊞1 member(s), 1 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus homom...	99.4	99.4	29%	2e-21	90.00%	295	XP_025552361.1
⊞3 member(s), 2 organism(s)	ascomycete fungi	fumarylacetoacetate hydrolase family protein [Aspergillus melleus]	99.0	99.0	29%	2e-21	86.00%	295	XP_045947258.1

Fig. 3. ORF32 protein similarity cluster. BLASTp 2025

The gene identified in *A. salvadorensis* is more related to metabolic functions of hydrolysis, typical of fumarylacetoacetate hydrolases, than to processes such as heme biosynthesis, mitochondrial transport of cofactors or direct degradation of plant polysaccharides. This analysis contributes to the functional characterization of the genome of the species and evidences the existence of enzymatic systems aimed at the transformation and recycling of organic compounds in their ecological environment.

Each protein characterized in databases is defined by a Unique Identifier, such as UniRef90_A2R528, which serves as a canonical reference within the repository. Included with this identifier are descriptive annotations (e.g., "Ferrochelatase"), which provide information about the biochemical function or major enzyme activity. In the case of ferrochelatase, its role in the final stage of heme group biosynthesis is recognized, through the insertion of the iron ion into protoporphyrin IX. In addition, these inputs are complemented by associated quantitative

metrics (e.g., 0.0961474), which reflect biological properties such as structural homology, genetic similarity, or potential expression levels.

Functional analysis based on these annotations is essential for comparative genomics, as it allows assigning biochemical roles to proteins and distinguishing those involved in key metabolic processes, such as heme synthesis or mitochondrial transport of cofactors. Comparison with proteins from other taxa, for example, the model fungus *Neurospora crassa* or even more distant organisms such as the turtle Chrysemys picta facilitates functional extrapolation through evolutionary homology. In turn, the interpretation of quantitative metrics helps to identify highly conserved proteins or those with outstanding biological activity.

In the context of *Aspergillus salvadorensis*, these strategies allow the identification of key proteins, the analysis of the distribution of numerical values to detect relevant conservations or divergences, and systematic comparisons with related species. This helps to elucidate the molecular basis of its biological properties and metabolic potential.

Among the most relevant proteins detected is ferrochelatase, which catalyzes the final step in the biosynthesis of the heme group. This reaction consists of the insertion of a ferrous ion (Fe^{2+}) into the protoporphyrin IX ring, generating heme, an essential cofactor in proteins involved in cellular respiration and electron transport in cytochromes.

Another important protein identified is the *mitochondrial* thiamine pyrophosphate transporter (Mitochondrial Thiamine Pyrophosphate Carrier 1). This transporter is involved in the movement of thiamine pyrophosphate, an active form of vitamin B1, into the mitochondrial matrix. In this cellular compartment, the molecule functions as an essential cofactor in various enzymatic reactions related to energy metabolism, especially in α-ketoacid decarboxylation processes.

The presence of annexins was also identified, a group of proteins that are characterized by their ability to bind to phospholipid-rich membranes in the presence of calcium. These proteins are involved in multiple cellular processes, including intracellular signaling, vesicle transport, and membrane repair.

Among the enzymes identified, *endo-β-1,4-glucanase* also stands out, *a*hydrolase capable of breaking β-1,4-glycosidic bonds present in structural glucans. In fungi, these enzymes are actively involved in cell wall remodeling, a process necessary during mycelium growth, hyphae formation, and spore germination.

These proteins reflect the existence of an active and complex metabolic network in *Aspergillus salvadorensis*, where processes related to energy production, the structural maintenance of the cell and adaptation to environmental factors are integrated. This functional repertoire evidences the ability of the fungus to survive in variable environments and to efficiently take advantage of the resources available in its environment.

Comparative analysis of these proteins using databases such as UniRef90 and sequence alignment tools also reveals a high degree of similarity to proteins present in other organisms. For example, some sequences show homology with hypothetical proteins described in *Neurospora crassa, suggesting* the evolutionary conservation of genes involved in fundamental cellular processes among different genera of filamentous fungi.

The observed similarities confirm that many of the proteins identified in *Aspergillus salvadorensis* participate in essential metabolic pathways highly conserved in eukaryotic organisms. This finding not only contributes to a better understanding of the molecular biology of this fungus, but also highlights its potential as a model of study in biotechnological research related to metabolism, enzyme production and environmental adaptation.

The phylogenetic tree analysis generated by BLAST indicates that the sequence of *Aspergillus salvadorensis* is located within a solid and highly populated clade of fungi of the phylum Ascomycota (Figure 4). In the visualization, the sequence of interest appears in a collapsed node that groups 52 closely related sequences, reflecting significant genetic proximity to other specimens of its genus or phylogenetically close species that are not individually deployed in the overview.

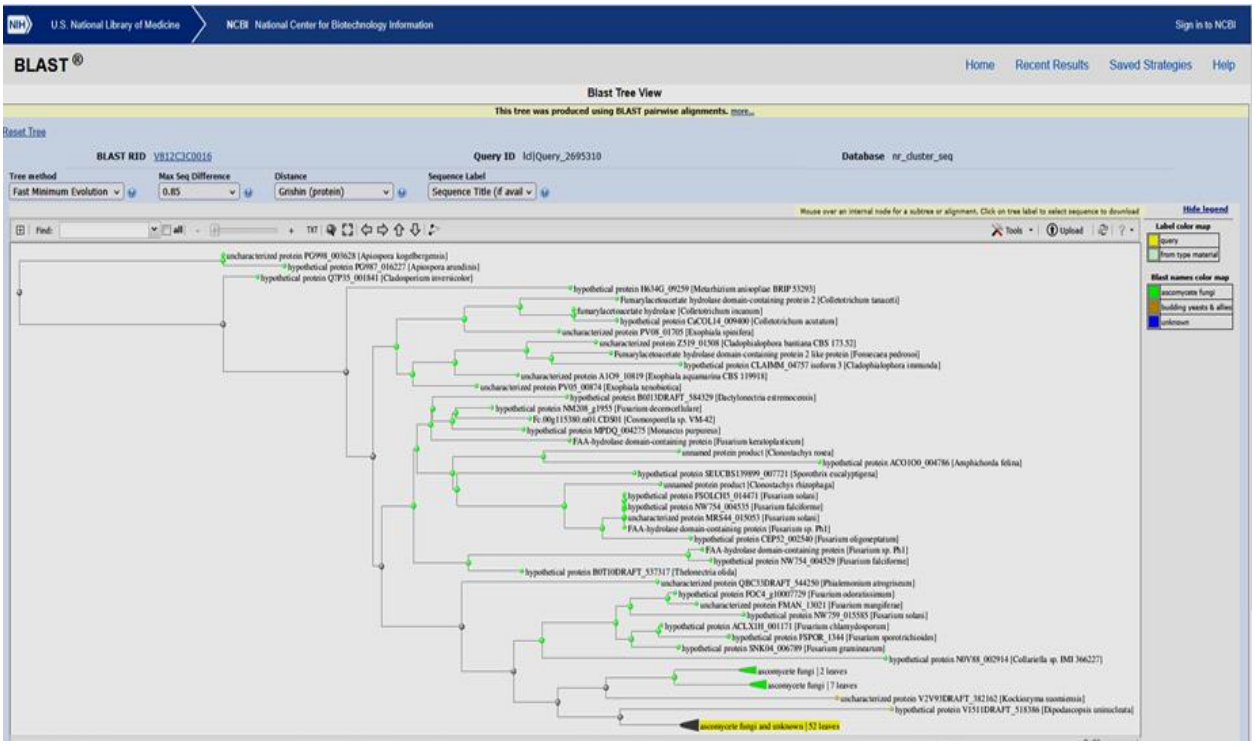


Fig. 4. Phylogenic Tree of *Aspergillus salvadorensis*. BLAST. 2025

The hierarchical structure of the tree shows that *A. salvadorensis* distances itself from other filamentous fungi present, such as the genera Fusarium, Colletotrichum and Exophiala. While the latter are distributed in the upper branches of the diagram, *A. salvadorensis* is located in the lower section, evidencing a clear evolutionary divergence with respect to these plant and environmental pathogens. The application of the Least Rapid Evolution method and Grishin distance confirms that, although they share common ancestors, the genetic identity of *A. salvadorensis* is distinctive and well-defined within its clade.

A relevant aspect of the analysis is the abundance of hypothetical or uncharacterized proteins in nearby branches. This suggests that, although phylogenetic technology allows *A. salvadorensis* to be located with great taxonomic precision, there is still a vast field of research on the specific biological function of these proteins shared with close relatives. Taken together, the tree positions this species within a solid core of ascomycetes, clearly differentiated from other more distant families of molds and yeasts.

When comparing *A. salvadorensis* with its better-known relative, *Aspergillus niger*, it is observed that both species are usually grouped into sister clades. *A. niger*, a cosmopolitan

organism widely used in industry for the production of citric acid and enzymes, shares with *A. salvadorensis* the morphology of dark spores, although the latter has a slightly differentiated molecular signature. This phylogenetic proximity reflects a similar genomic architecture, adapted to the degradation of complex organic matter and survival in environments with low water availability, although genetic differences allow *A. salvadorensis* to be recognized as an independent taxonomic entity, with a particular profile of secondary metabolites.

From an ecological and functional perspective, while genera such as *Fusarium* tend to specialize as aggressive plant pathogens, the clade that integrates *A. salvadorensis* and *A. niger* adopts a saprophytic or opportunistic pathogen lifestyle. The Grishin distance in the phylogenetic tree acts as a molecular chronometer, indicating that *Aspergillus* has perfected mechanisms of resistance to osmotic and thermal stress, differentiating them from phytopathogenic fungi such as Colletotrichum or Metarhizium.

The analysis of hypothetical proteins present in this clade reveals considerable biotechnological potential. Although these sequences do not yet have an experimentally validated function, they could represent enzymatic variants optimized for biomass degradation, including endoglycanases, or catalysts specialized in the synthesis of secondary metabolites of high industrial value. In this context, some of these proteins could be involved in metabolic pathways dependent on thiamine pyrophosphate (TPP), a crucial cofactor in the decarboxylation of ketoacids, essential processes for the production of organic acids and the efficient obtaining of energy in saprophytic fungi.

Phylogenetic analysis confirms that *Aspergillus salvadorensis* belongs to a well-defined clade of ascomycetes, closely related to *A. niger*, and with a genomic architecture that supports its ability to degrade complex organic compounds and adapt to particular environmental conditions. The presence of hypothetical proteins suggests promising areas for future functional and biotechnological studies, offering a window into new enzymes and metabolites with potential applications in industry and scientific research.

Deepening the analysis of the hypothetical proteins present in *Aspergillus salvadorensis* allows us to transform generic tags into concrete functional tools. Examination of conserved domains within these proteins can reveal molecular fingerprints linking them to the production of industrial enzymes, such as pectinases and proteases, which are characteristic of the genus Aspergillus. Thus, the branches highlighted in the phylogenetic tree not only represent genetic sequences, but also function as a map of opportunities for mycological research, pointing out possible routes towards the discovery of biochemical capacities not yet explored in the Salvadoran ecosystem.

The phylogenetic position of *A. salvadorensis*, close to consolidated industrial clades such as that of *Aspergillus niger*, suggests a highly specialized metabolic potential in the degradation of complex polymers. The proximity of several hypothetical proteins to the consulted sequence indicates the existence of an enzymatic machinery that has not yet been characterized, with probable secretion of pectinases and proteases adapted to specific environmental conditions. This enzymatic versatility is crucial for the hydrolysis of the plant cell wall and the assimilation of protein nitrogen, reinforcing both the ecological role of the species in its habitat and its biotechnological value.

Additionally, the identification of sequences related to enzymes dependent on essential cofactors, such as thiamine pyrophosphate, suggests an optimized efficiency in oxidative metabolic pathways. These enzymes play critical roles in keto acid decarboxylation and other energy reactions, positioning *A. salvadorensis* as a promising candidate for fermentation processes, secondary metabolite production, and industrial applications based on the biotransformation of organic compounds.

In the regulatory context, the TAF(II) protein of *A. salvadorensis* acts as an indispensable mediator of basal transcription. These proteins contain specific domains, such as histone-fold domains, which allow them to form dimers and form the core of the TFIID multiprotein complex. In a fungus with high secretory capacity, the efficiency of TAF ensures that enzyme-coding genes such as pectinases and proteases are transcribed in a coordinated and massive manner when the fungus colonizes an organic substrate. This evidences the integration between transcriptional regulation and metabolic capacity, a key trait that underpins both the environmental adaptability and the biotechnological potential of this species.

The phylogenetic and functional analysis of *A. salvadorensis* reveals an organism with a genome structured to maximize its metabolic and regulatory efficiency, with enzyme machinery specialized in the degradation of plant biomass and efficient cofactor-dependent metabolic pathways. This profile combines ecological adaptations with potential biotechnological applications, from the production of industrial enzymes to the synthesis of secondary metabolites of interest, positioning this species as a promising resource for molecular biology studies and industrial applications under specific environmental conditions.

DISCUSSION

The bioinformatic analysis of the protein repertoire of *Aspergillus salvadorensis* shows a complex functional organization that integrates essential metabolic processes, gene regulation and environmental adaptation mechanisms. The characterization of proteins using reference databases such as UniRef90 allowed the identification of enzymes and transporters involved in fundamental metabolic pathways, suggesting that the genome of this species harbors a highly conserved set of genes related to energy production, cofactor biosynthesis and cellular structural remodeling.

Among the most relevant proteins is ferrochelatase, a key enzyme in the biosynthesis of heme. This protein catalyzes the insertion of a ferrous ion into protoporphyrin IX, generating the heme group, an indispensable cofactor for numerous proteins involved in redox processes and in the transport of electrons within the mitochondrial respiratory chain. The presence of this enzyme indicates that *A. salvadorensis* possesses an efficient metabolic machinery to sustain aerobic respiration and energy production, while maintaining precise control over iron metabolism, essential to avoid toxicity due to excessive accumulation (Dailey *et al.*, 2017).

Another protein of interest is the thiamine pyrophosphate mitochondrial transporter, responsible for mobilizing thiamine pyrophosphate, the active form of vitamin B1, into the mitochondrial matrix. This cofactor is essential for the activity of several enzymes involved in the oxidative decarboxylation of α-ketoacids, critical steps for acetyl-CoA formation, and the efficient functioning of the Krebs cycle. Its presence suggests that *A. salvadorensis* has optimized mechanisms to regulate its energy metabolism, which contributes to its ability to adapt to conditions with variable nutrient availability (Marobbio *et al.*, 2002).

Regarding cell structure, hydrolytic enzymes such as endo-β-1,4-glucanase, belonging to the glycosyl hydrolase group, were identified. These enzymes break glycosidic bonds present in structural polysaccharides, such as fungal cell wall glucans, participating in wall remodeling during mycelium growth, cell differentiation, and spore germination. The presence of these glucanases indicates that the fungus has a dynamic enzyme system capable of modifying its cellular architecture to adapt to environmental changes or different phases of its life cycle (Gavande and Goyal, 2023).

The values associated with these proteins 29.4366 for ferrochelatase, 31.4809 for the thiamine pyrophosphate transporter, and 31.2345 for endo-β-1,4-glucanase probably represent parameters derived from sequence

similarity analyses, such as alignment scores or identity indices. These indicators allow estimating the evolutionary conservation of proteins with respect to reference sequences in international databases. In general, higher values reflect a greater match with previously characterized proteins, facilitating the functional assignment of newly identified genes. In the case of *A. salvadorensis*, the results suggest a high similarity with proteins from other species of the genus *Aspergillus* and related filamentous fungi, which reinforces the idea that these metabolic and structural functions are evolutionarily conserved within the clade of ascomycetes. The findings highlight that *A. salvadorensis* has a genome that integrates metabolic, regulatory, and structural systems in a coordinated manner, ensuring its environmental adaptation, energy efficiency, and biotechnological potential for the production of industrial enzymes and secondary metabolites (Vásquez, 2026).

The analysis of the genome and proteome of *Aspergillus salvadorensis* reveals an organism with a complex functional organization, capable of integrating essential metabolic processes, precise gene regulation and environmental adaptation mechanisms. The characterization of its proteins using reference databases, such as UniRef90, allowed the identification of enzymes and transporters involved in fundamental metabolic pathways, suggesting that the genome of this species contains a highly conserved set of genes related to energy production, cofactor biosynthesis and cellular structural remodeling. Among the most relevant proteins is ferrochelatase, an enzyme that catalyzes the insertion of a ferrous ion into protoporphyrin IX to generate heme, an indispensable cofactor for proteins involved in redox processes and in the mitochondrial respiratory chain. Its presence indicates that *A. salvadorensis* maintains an active system of iron metabolism and heme biosynthesis, crucial for efficient energy generation and growth in environments rich in organic matter.

Additionally, the mitochondrial thiamine pyrophosphate transporter was identified, responsible for mobilizing the active form of vitamin B1 towards the mitochondrial matrix, where it acts as a cofactor in the oxidative decarboxylation of α-ketoacids, contributing to the production of acetyl-CoA and the functioning of the Krebs cycle. The presence of this transporter indicates that the fungus has efficient mechanisms to regulate its energy metabolism and adapt to variations in nutrient availability. Endo-β-1,4-glucanase, belonging to the glycosyl hydrolase group, suggests a dynamic system of cellular structural remodeling, allowing the cell wall to be modified during mycelium growth, spore germination and cell differentiation in the face of environmental changes.

The identification of TAF(II), a factor associated with the TFIID complex, evidences a highly regulated transcriptional system. This protein facilitates promoter recognition, recruits RNA polymerase II, and coordinates gene expression in response to environmental cues, including nutrient availability, oxidative stress, and physicochemical variations. Its activity ensures the efficient transcription of pectinase and protease coding genes, essential for the degradation of plant polymers and the assimilation of protein nitrogen, which highlights the ability of *A. salvadorensis* to colonize organic substrates and maintain a highly efficient adaptive metabolism.

Comparative analyses showed similarities with proteins of *Neurospora crassa*, indicating the conservation of critical metabolic pathways in filamentous fungi and other eukaryotes, and coincidences with more distant organisms such as the turtle *Chrysemys picta*, attributable to the conservation of universal structural domains involved in molecular transport, signaling and energy metabolism. This similarity indicates that many fundamental metabolic pathways are evolutionarily conserved between different species of ascomycete fungi, reflecting the functional importance of these genes in cell biology and ecological adaptation (Galagan *et al.*, 2003). Phylogenetically, *A. salvadorensis* is located in a robust clade of ascomycetes, close to *Aspergillus niger,* sharing saprophytic and enzymatic capacities to degrade complex

organic matter, although it presents differences in its molecular signature that distinguish it as an independent species. The presence of hypothetical proteins in adjacent clades suggests potential for discovering new enzymes and industrial metabolites not yet characterized.

The analysis also detected proteins associated with antifungal resistance, such as those linked to fluconazole, indicating that the fungus could tolerate compounds present in its natural environment. TAF(II)-mediated transcriptional regulation could facilitate the activation of genes encoding efflux pumps, detoxifying enzymes, or cell wall proteins, contributing to survival under chemical stress conditions. In addition, this regulation allows *A. salvadorensis* to respond to environmental stress, nutrient limitation and pH variations, ensuring its growth and viability in its natural habitat.

Genomic and proteomic analysis shows that *Aspergillus salvadorensis* has a sophisticated metabolic network that combines the ability to produce energy, synthesize cofactors, degrade complex polymers and regulate gene expression in an adaptive manner. The presence of ferrochelatase, cofactor transporters, hydrolytic enzymes, TAF(II) and hypothetical proteins highlights its ecological and biotechnological potential, positioning this species as a promising candidate for studies of fungal physiology, industrial biotechnology and exploration of new enzymes and metabolites of interest.

The proteomic analysis of *Aspergillus salvadorensis* reveals a repertoire of proteins that reflect both its metabolic capacity and its regulatory sophistication and environmental adaptation. Among the proteins identified is the mitochondrial thiamine pyrophosphate transporter, known as Thiamine Pyrophosphate Carrier 1, which mobilizes thiamine pyrophosphate into the mitochondrial matrix, where it acts as a cofactor in essential α-ketoacid decarboxylation reactions, which are essential for the tricarboxylic acid cycle and carbohydrate metabolism. Its presence indicates that *A. salvadorensis* has efficient mechanisms to regulate energy metabolism and produce energy from different sources of carbon in its environment.

Likewise, proteins of the annexin family were identified, capable of binding to phospholipid membranes in the presence of calcium. These proteins participate in intracellular signaling, exocytosis and membrane repair, contributing to calcium homeostasis and plasma membrane integrity, critical aspects for survival under conditions of environmental stress. In fungal organisms, annexins also contribute to maintaining calcium homeostasis and preserving the structural integrity of the cell in the face of environmental changes or stress conditions, which highlights their role in the physiological stability of the organism (Gerke and Moss, 2002). In parallel, the detection of the enzyme endo-β-1,4-glucanase D evidences the ability of the fungus to degrade glucans from the cell wall, allowing dynamic mycelium remodeling, spore germination and cell differentiation. The activity of these glucans is essential for hyphal growth and structural adaptation to different environments, since they are primary components of the fungal cell wall.

The numerical values associated with proteins such as ferrochelatase, the mitochondrial transporter of thiamine pyrophosphate and endo-β-1,4-glucanase reflect metrics derived from sequence similarity analyses, indicating a high degree of evolutionary conservation with respect to homologous proteins in other filamentous fungi. This conservation suggests that the catalytic and structural functions of these proteins have remained stable throughout evolution, ensuring the metabolic and adaptive efficiency of the organism.

On the other hand, the TAF(II) protein, a subunit of the TFIID complex, essential in the initiation of RNA polymerase II-dependent transcription, was identified. This complex recognizes specific promoters, such as the TATA box, and facilitates the assembly of the transcriptional machinery, ensuring the synthesis of messenger RNA. TAF subunits serve additional functions, including interaction with activating or repressor factors and chromatin remodeling, which regulates DNA

accessibility and allows selective activation of genes in response to environmental cues. This regulation is critical to coordinate the expression of metabolic, enzymatic and adaptive genes, such as those coding for pectinases, proteases and proteins related to antifungal resistance, ensuring the survival of the fungus in the face of nutrient variations, oxidative stress and changing physicochemical conditions.

The functional and comparative analysis of the proteome of *Aspergillus salvadorensis* reveals a highly adaptive organism, capable of integrating complex metabolic processes, structural remodeling and sophisticated transcriptional regulation, reflecting its ability to survive in variable environments and its biotechnological potential in the production of industrial enzymes, secondary metabolites and resistance systems against chemical compounds (Vásquez, 2026).

In *Aspergillus salvadorensis*, transcriptional regulation mediated by complexes such as TFIID is critical to control key physiological processes, such as sporulation, mycelial growth, and the production of secondary metabolites. These metabolites include bioactive compounds of high biotechnological relevance, such as antibiotics, industrial enzymes and mycotoxins. The presence of proteins related to TAF(II) suggests that this organism has a regulatory system capable of coordinating the expression of genes involved in these metabolic functions. In addition, TAF(II) activity could be associated with the regulation of environmental stress response mechanisms, including oxidative stress, nutrient limitation or exposure to toxic compounds. In many fungi, the activation of genes involved in the stress response depends on transcriptional complexes sensitive to intracellular and extracellular signals, allowing the organism to adjust its metabolism quickly and maintain cellular homeostasis (Lara-Martínez *et al.*, 2025; Yaakoub *et al.*, 2022).

Another relevant aspect of the analysis is the possible presence of antifungal resistance mechanisms, particularly against compounds such as fluconazole. In pathogenic fungi, this resistance is usually related to the activation of genes that encode flux pumps, detoxifying enzymes or proteins involved in cell membrane biosynthesis, allowing the fungus to reduce the intracellular concentration of the drug or modify its site of action. The regulation of these genes may depend on transcription factors such as TAF(II), suggesting that this type of regulatory protein could play an indirect role in tolerance to antifungals.

Genomic and proteomic analysis of *A. salvadorensis* also revealed a repertoire of key metabolic enzymes, structural proteins, and hydrolytic enzymes capable of degrading complex organic compounds. These include endo-β-1,4-glucanases, which are involved in cell wall remodeling during hyphal growth and spore germination, and thiamine pyrophosphate mitochondrial transporters, which are essential for energy generation through α-ketoacid metabolism. Likewise, annexins, proteins that regulate calcium homeostasis and maintain the integrity of the plasma membrane in the face of environmental stress, and ferrochelatase, involved in the biosynthesis of heme and the efficient functioning of the mitochondrial respiratory chain, were identified. These findings reflect a sophisticated cellular system, capable of integrating energy metabolism, structural remodeling, and transcriptional regulation to adapt to varying environmental conditions.

From a biotechnological perspective, the molecular properties identified in *A. salvadorensis* suggest that this species could be a valuable resource for industrial and environmental applications. Hydrolytic enzymes produced by fungi of the genus Aspergillus have been widely used in the food, pharmaceutical and energy industries for the production of industrial enzymes, biofuels and biomass products. In addition, the ability to degrade polymers and complex biomass positions these organisms as promising candidates for bioremediation and biological recycling of organic and synthetic waste. Overall, the protein repertoire of *A. salvadorensis* shows remarkable adaptive capacity, versatile metabolism and considerable biotechnological potential,

reflecting its ecological success and relevance for human applications.

CONCLUSION

Genomic and proteomic analysis reveals the presence of a complex functional network that integrates metabolic, structural, and regulatory processes. The identification of enzymes related to heme biosynthesis, mitochondrial transporters, structural hydrolases, and transcription factors indicates that this organism possesses highly specialized molecular machinery to adapt to variable environments.

The similarities observed with proteins from other filamentous fungi reflect the evolutionary conservation of essential metabolic pathways, while the presence of proteins associated with antifungal resistance suggests possible mechanisms of adaptation against antimicrobial compounds.

The results highlight the potential of *Aspergillus salvadorensis* as a study model for research related to fungal metabolism, gene regulation and production of enzymes of biotechnological interest. It has a highly integrated metabolic network that combines energy, structural and regulatory processes. The presence of enzymes involved in heme biosynthesis suggests the existence of efficient mechanisms of cellular respiration and iron metabolism, fundamental processes for energy production in aerobic organisms.

Likewise, the identification of mitochondrial transporters related to cofactor metabolism indicates a close connection between metabolic regulation and mitochondrial functioning. This organelle plays a central role in energy generation and in regulating cell metabolism.

The hydrolytic enzymes identified in the genome also suggest that this organism possesses a remarkable ability to remodel its cell wall and possibly to degrade complex polymers present in its environment. This characteristic is common in filamentous fungi that inhabit environments rich in organic matter, where biomass degradation is a key strategy for obtaining nutrients.

The identification of proteins associated with antifungal resistance mechanisms also raises important questions about the ability of this organism to tolerate antimicrobial compounds present in its natural environment. These mechanisms could be related to membrane transport systems or to modifications in the enzymes targeted by antifungals.

The presence of transcription-regulating factors such as TAF(II) suggests that *Aspergillus salvadorensis* has a sophisticated gene control system that allows it to respond efficiently to environmental changes. This type of regulation can influence the expression of genes related to metabolism, growth and production of secondary metabolites.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

GENERATIVE AI STATEMENT

This study used Generative AI tools in data re-organization. We confirm that all AI-assisted processes were critically reviewed by the author’s to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the author’s.

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