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Mechanisms of Antifungal Resistance of ABC Transporator Genes in *Aspergillus uessalvadorensis* (2025)

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ABSTRACT **Original Research Article**

The present study on *Aspergillus uessalvadorensis* focuses on the characterization of the molecular mechanisms that give it a high adaptive capacity in highly toxic environments. Through a comprehensive genomic workflow, which included sequencing using high-throughput platforms (Illumina), expression analysis using qPCR, and functional annotation in bioinformatics tools such as InterProScan, UniProtKB, tBLASTn, UniRef90, MetaCyc, EggNOG, and KEGG, it was possible to establish a robust relationship between genomic sequencing and the organism's adaptive strategies. The core of this fungus's resistance lies in the expansion and specialization of genes associated with multidrug efflux systems. In particular, the superfamily of ABC transporters (ATP-Binding Cassette), highly specialized membrane proteins that function as active expulsion pumps, stands out. These are composed of transmembrane domains (TMDs), responsible for substrate recognition and transport, and cytoplasmic nucleotide-binding domains (NBDs), responsible for ATP hydrolysis that drives the conformational change necessary for transport. In addition, the presence of transporters belonging to the Major Facilitator Superfamily (MFS) was identified, which operate through electrochemical gradients. This coexistence suggests a strategy of functional redundancy and specialization: while ABC systems participate in the active expulsion of more complex compounds, MFS transporters contribute to cellular homeostasis and the handling of simpler metabolites. A relevant finding is the identification of a large protein (~2450 amino acids) that contains conserved domains associated with transport and regulation. In addition, genes encoding components of the DNA repair system, such as ABC excinuclease subunits, were detected. Although these are not directly involved in the efflux of toxic compounds, they play a crucial role in repairing damage induced by oxidative stress, UV radiation, or other environmental agents. This shows a comprehensive defense system that combines exclusion (eflux) mechanisms with tolerance and genomic repair strategies. From an ecological perspective, factors such as exposure to soils with a high concentration of heavy metals and the intensive use of fungicides could have exerted selective pressure, favoring the expansion of these transport systems. In this sense, the identified transporters would not only be involved in resistance to antifungal compounds, but also in the regulation of ionic homeostasis and adaptation to extreme environments. In mechanistic terms, the operation of ABC transporters can be described as a cyclical process: after the entry of a potentially toxic compound into the cell, the substrate is recognized by the transmembrane domains. Subsequently, the NBD domains hydrolyze ATP (ATP → ADP + Pi), generating the energy necessary to induce a conformational change in the protein, allowing the active expulsion of the compound to the outside of the cell. This mechanism is one of the main barriers to antimicrobial agents. In conclusion, the genomic profile of *A. uessalvadorensis* reveals a complex and multifactorial molecular architecture oriented to survival in adverse environments. However, although genomic evidence suggests a high potential for resistance, it is imperative to validate these findings through experimental studies,

including gene expression analysis (qPCR) and phenotypic susceptibility assays. These approaches will confirm the functional impact of efflux systems and their relevance in clinical and environmental contexts.

Keywords: ABC Multridrog Transporter, *Aspergillus uessalvadorensis*, MFS Major Facilitator Superfamily, Illumina, InterProScan.

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Introduction

Antifungal resistance is one of the main contemporary challenges in medical and environmental microbiology, especially in fungi of the genus *Aspergillus*, which includes species of high clinical, agricultural and ecological relevance. These filamentous fungi exhibit remarkable adaptive plasticity that allows them to survive in environments with high chemical pressure, including exposure to natural antimicrobial compounds and synthetic drugs. Among the molecular mechanisms that underpin this ability are membrane transport systems, particularly those belonging to the ATP-Binding Cassette (ABC) superfamily, which play a fundamental role in the active expulsion of toxic substances out of the cell (Prasad & Goffeau, 2012).

ABC transporters are transmembrane proteins that use the energy derived from ATP hydrolysis to mobilize a wide variety of substrates across the plasma membrane. In fungi, these systems function as multi-drug efflux pumps, capable of reducing the intracellular concentration of antifungals such as azoles, polyenes and echinocandins, thus reducing their therapeutic efficacy. In species such as *Aspergillus fumigatus*, overexpression of genes encoding ABC transporters has been shown to contribute significantly to the resistance phenotype, particularly in the face of prolonged azole treatments (Verweij et al., 2016). This phenomenon not only has clinical implications, but also ecological ones, as it favors the survival of the fungus in niches rich in secondary metabolites produced by competing microorganisms.

In addition, antifungal resistance does not depend on a single system, but results from the interaction of complex molecular networks that include transporters of the Major Facilitator Superfamily (MFS), DNA repair systems and oxidative stress response mechanisms. This functional integration gives the organism an adaptive advantage in the face of adverse environmental conditions, allowing it to maintain cellular homeostasis and preserve genomic integrity (Cowen et al., 2015).

In this context, the analysis of ABC transporter genes in *Aspergillus uessalvadorensis* acquires special relevance to understand their potential for antifungal resistance and their ability to adapt to extreme environments. The identification and molecular characterization of these genes provides a solid basis for future functional investigations aimed at evaluating their expression, regulation and contribution to the resistant phenotype, both in clinical and environmental contexts.

Material and Methods

The genomic localization procedure is based on a comparative bioinformatics approach that integrates multiple tools and specialized databases. Initially, reference sequences of transporters belonging to the ABC (ATP-Binding Cassette) and MFS (Major Facilitator Superfamily) superfamilies were used, obtained from UniProtKB by means of UniRef90 clusters previously identified in the molecular characterization of the organism. (SEE Table I)

The identification of the gene sequences was carried out by consulting repositories of the National Center for Biotechnology Information, specifically the BioProject PRJNA1306032, corresponding to the genomic assembly of *Aspergillus uessalvadorensis* obtained by means of next-generation sequencing (NGS) technologies. For the precise localization of the coding genes, the tBLASTn algorithm was used, which allows aligning protein sequences against translated genomes. A threshold of statistical significance was established with expectation values (E-value) less than (1 \times 10^{-10}), guaranteeing the identification of relevant homologies and minimizing random coincidences.

Once the gene loci were identified, the genomic DNA sequences were extracted, including potentially regulatory flanking regions, such as promoters and terminators. Subsequently, these sequences were analyzed using functional databases such as KEGG (Kyoto Encyclopedia of Genes and Genomes) and MetaCyc, which allowed the reconstruction of metabolic pathways associated with the transport of compounds and the identification of possible substrates, including drugs and secondary metabolites.

The structural validation of the identified genes was carried out using automatic annotation tools such as InterProScan, confirming the presence of conserved domains characteristic of multidrug transporters, such as transmembrane domains (TMD) and nucleotide binding domains (NBD).

The genomic analysis was based on high-quality data generated by the sequencing platform of Macrogen Inc. (South Korea). To do this, the extraction of genomic DNA (gDNA) from pure cultures was carried out, ensuring its molecular integrity. The preparation of libraries and massive

sequencing allowed obtaining a robust dataset, whose quality was evaluated by parameters such as GC content (~49.7%), facilitating the assembly of genomic scaffolds containing the genes of interest.

The objective of the investigation is to find in the sequence according to the report of Macrogeninc, if there are indications in the ABC and MTS sequence. Clarification of antifungal susceptibility analysis is for future research. The number that references UniRef90 refers to the frequency found in Table I. The study of the qDNA sequence is documented in the publication of the *Aspergillus uessalvadorensis* DNA article (Plant Volume 13, Issue 1,/j. plant.20251301.11) and registered in GenBank (NCBI): Registered under BioProjects PRJNA1306032 and PRJNA1303219 or also called *Aspergillus salvadorensis* (Registration in Fungal Name (FN): 573057). (Vásquez, 2025). It is also clarified that the AI image in Fig 3 is for academic and physiological illustrative purposes of what was found in the sequences. Expression analysis using qPCR will be for future research specifically of ABC and MTS.

Finally, the workflow integrated the genomic annotation results with assumed gene expression data in the sequence reported by Macrogeninc, allowing the construction of a functional model of multidrug resistance. This model provides a predictive basis for the phenotypic behavior of *A. uessalvadorensis* against different classes of antifungals, based on the molecular and functional architecture of the identified transporters.

Results

Table I. DNA sequence analysis of antifungal transporters present in *Aspergillus uessalvadorensis*. MACROGEN INC. 2026

COG ID *Aspergillus*	Orthology	MetaCyc/EggNOG/KEGG/Uniref90/K
UniRef90_G7XHP0	ABC multidrug transporter	31.7238
UniRef90_G7XIT3	ABC transporter family protein	1.46734
UniRef90_G7XLR5	MFS transporter	29.7765
UniRef90_G7XQH9	ABC multidrug transporter	31.4713
UniRef90_S3B4G4	Sugar porter (SP) family MFS transporter	0.74188
UniRef90_G7XXM7	ABC multidrug transporter Mdr2	2.41132
UniRef90_X0P1V3	Peptide ABC transporter ATP-binding protein	0.789705
UniRef90_W1XKU8	ABC transporter, ATP-binding protein (Fragment)	0.113171
COG0488	(ABC) transporter	0.608174
COG0559	(ABC) transporter	3.59562
COG1119	(ABC) transporter	1.52481
Environmental Information Processing 39	ABC transporters	4
K01992 157	ABC-2 type transport system permease protein	0.517629
K03701	Excinuclease ABC subunit A	0.262557
K03702	Excinuclease ABC subunit B	0.413693

The analysis of the data presented in Table I reveals a high representation of proteins associated with membrane transport systems, particularly transporters of the ABC (ATP-Binding Cassette) and MFS (Major Facilitator Superfamily) superfamilies. This pattern is highly relevant, since both systems are widely involved in antifungal resistance mechanisms, especially through the active expulsion of toxic compounds. The numbers indicate the frequency found by each UniRef90.

The recurrent presence of multidrug ABC transporters suggests a central role of these systems in the physiology of *Aspergillus uessalvadorensis*. These transmembrane proteins use the energy derived from ATP hydrolysis to export a wide variety of substrates, including antifungals. In fungi of the genus *Aspergillus*, overexpression or duplication of ABC genes has been associated with multidrug resistance (MDR) phenotypes, by reducing the intracellular concentration of drugs and, therefore, their efficacy.

In addition, transporters of the MFS superfamily are identified, including sugar-porter proteins. Although traditionally associated with the transport of sugars, many members of this family are also involved in the export of xenobiotic compounds. The coexistence of ABC and MFS transporters suggests a functional redundancy strategy that increases the body's adaptive capacity in the face of environmental chemical stress or pharmacological pressure.

Additionally, the detection of components such as ABC excinuclease (A and B subunits) indicates the presence of highly conserved DNA repair mechanisms. These systems, although they do not participate directly in the efflux of drugs, contribute to cellular tolerance against genetic damage

induced by oxidative stress or radiation, reinforcing survival in adverse conditions.

From a structural and functional point of view, ABC transporters have transmembrane domains (TMDs), responsible for substrate recognition and translocation, and nucleotide-binding domains (NBDs), responsible for ATP hydrolysis. This process drives conformational changes that allow the active expulsion of molecules to the outside of the cell. On the other hand, MFS transporters, characterized by approximately 12 transmembrane helices, operate through electrochemical gradients, generally of protons, facilitating passive or coupled transport.

The identification of the UniRef90_G7XLR5 entry as an MFS transporter confirms the presence of this type of protein in the analyzed genome. Importantly, functional MFS transporters typically have lengths between 400 and 600 amino acids, which allows their correspondence with previously identified short ORFs, such as ORF35, to be ruled out. This reinforces the need to analyze complete sequences and their domain architecture for a correct functional assignment.

In relation to UniRef90 identifiers, these represent clusters of streams with at least 90% identity. The association of multiple inputs with *Aspergillus* kawachii proteins suggests a high degree of evolutionary conservation in membrane transporters. However, it is important to specify that this grouping indicates significant homology, but not necessarily absolute functional identity, so possible variations in regulation and activity should be considered.

On the other hand, KEGG functional codes (such as K01992, K03701, and K03702) allow these genes to be contextualized within specific metabolic pathways. In particular, K01992 corresponds to ABC-type transport systems involved in compound mobilization, while K03701 and K03702 are associated with DNA repair systems using excinucleases, which are essential for genomic stability.

The observed genetic profile suggests a high potential for antifungal resistance mediated mainly by efflux mechanisms and complemented by cell repair systems. However, it is important to emphasize that the presence of these genes does not necessarily imply an active resistant phenotype. Functional validation requires additional analyses, including gene expression studies and phenotypic assays for antifungal susceptibility.

From Table I the UniRef90 are identified and then passed to the UniProtKB program and then to tBLASTn, the result is:

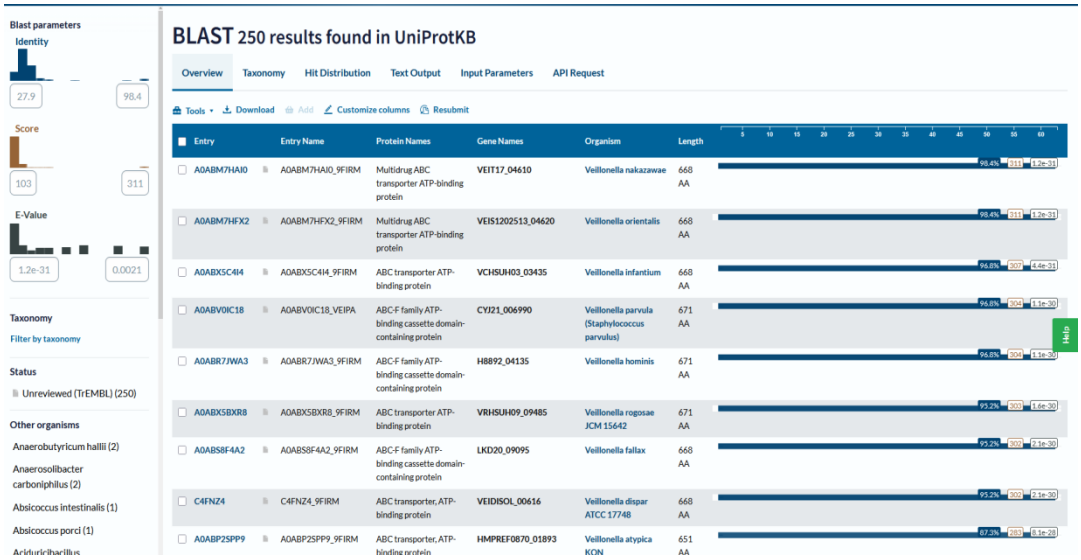


Fig 1. Result of sequence analysis *A. uessalvadorensis*. tBLASTn. 2025

In Fig 1. The analysis of the results obtained by BLAST reveals a significant relationship between the sequence studied and proteins annotated in bacteria of the genus Veillonella, with identities greater than 95%. At first glance, this finding could suggest a bacterial origin of the sequence; However, this interpretation should be approached with caution.

The transporters of the ABC superfamily are one of the oldest and most evolutionarily conserved protein families, present in both prokaryotes and eukaryotes, including fungi of the genus *Aspergillus*. Therefore, the high similarity observed in the BLAST results does not necessarily indicate a direct bacterial origin, but rather the structural conservation of essential functional domains.

In particular, the alignment shows a high conservation in the nucleotide binding domains (NBD), which contain characteristic motifs such as Walker A and Walker B, responsible for the hydrolysis of ATP. These domains represent the functional core of active transport and are usually highly conserved even among evolutionarily distant organisms.

On the other hand, the regions corresponding to the transmembrane domains (TMDs) show greater variability. This divergence is consistent with the functional

specialization of each organism, as these regions determine the specificity of the substrates transported. In fungi such as *Aspergillus uessalvadorensis*, ABC transporters are usually adapted to the recognition and expulsion of antifungal compounds and complex metabolites, while in bacteria such as *Veillonellanakazawae* they may be specialized in other types of molecules.

From an evolutionary perspective, this similarity can be explained by the preservation of a highly efficient ancestral transport system, rather than by a shared recent origin. Alternatively, in some specific cases, horizontal gene transfer could also be considered, although this hypothesis would require additional evidence, such as robust phylogenetic analyses and study of the genomic context.

It is important to note that tools such as UniRef90 group sequences with at least 90% identity, but this does not necessarily imply complete functional identity or common taxonomic origin. Automatic annotation can favor matches with organisms that are better represented in databases, such as bacteria, which introduces bias into the results.

The data in Table I suggest that the sequence analyzed belongs to a highly conserved ABC transporter, whose functional architecture is shared between bacteria and fungi. However, the definitive taxonomic assignment should be based on a broader analysis that includes genomic context, full protein length, domain prediction, and phylogenetic reconstruction.

The protein sequence *of Aspergillus uessalvadorensis* compared to Bacteria, is as follows:
>UniRef90_G7XHP0 | Aspergillus multidrugtransporter (Referencia Tabla)
MGRESLVRLFRYSSYDLVLADGRVLRALRGLSVPFGR
TVALVGPNGAGKSTLLALVAGLL
EPTSGEVLLDGRDLATYDHRLRHLRGVIVPQESVLFND
TIAENIAYGRAGATREEIARAA
KMANAHEFILSFPQQYNVLVGERGVLSSGGQKQRIAIA
RALVNNPKILLLDEATSALDAE
... CONTINUE
>A0ABM7HAI0 | Veillonellanakazawae ABC transporter (Resultado BLAST)
MIRLKNVSIKFPNVDFEVLDGINLTIHKGEITAIIGPNGS
GKSTLLNIIGGLDKPDSGEL
YIDGKNINDLDMKELRKKIGIVFQHFNLFPHLTVLENII
FGPKIKMKDREKIDRVVEMAL
KLKIRNHKFIDRLSGGQKQRVAIARALANNPKIILLDEA
TSALDSNSEKIVQDALDKLMA
... CONTINUE

The alignment of the sequences reveals the presence of highly conserved regions in the central zone of the protein, which is characteristic of transporters of the ABC superfamily. Although the "GGQKQR" motif is identified as a conserved region in the analyzed sequences, it is important to note that the classical molecular signatures of nucleotide-binding domains (NBDs) on ABC transporters correspond mainly to well-established motifs such as Walker A (GxxxxGKST), Walker B, and the signature motif LSGGQ. Thus, the observed motif could be part of a closely conserved or structurally derived region, but it does not in itself constitute the universal signature of the system.

The high conservation of these domains explains why, when searching databases, the sequences show significant similarities to proteins from phylogenetically distant organisms, such as bacteria of the genus Veillonella. This similarity does not necessarily imply a bacterial origin of the sequence analyzed, but reflects the evolutionary conservation of ATP-dependent transport mechanisms.

In this context, the NBD domains represent the functional core of the transporter, responsible for the binding and hydrolysis of ATP, a process that drives the conformational changes necessary for active transport. This "energy machinery" has remained highly conserved since the common ancestors of prokaryotes and eukaryotes, including fungi of the genus Aspergillus.

As for obtaining DNA sequences, the bioinformatics procedure is based on the use of access identifiers in specialized databases. From an identifier such as UniRef90_G7XHP0, it is possible to access the corresponding entry in UniProtKB, where the functional annotation of the protein is located. Within this tab, the Sequence databases section provides direct links to repositories such as GenBank, hosted at the National Center for Biotechnology Information.

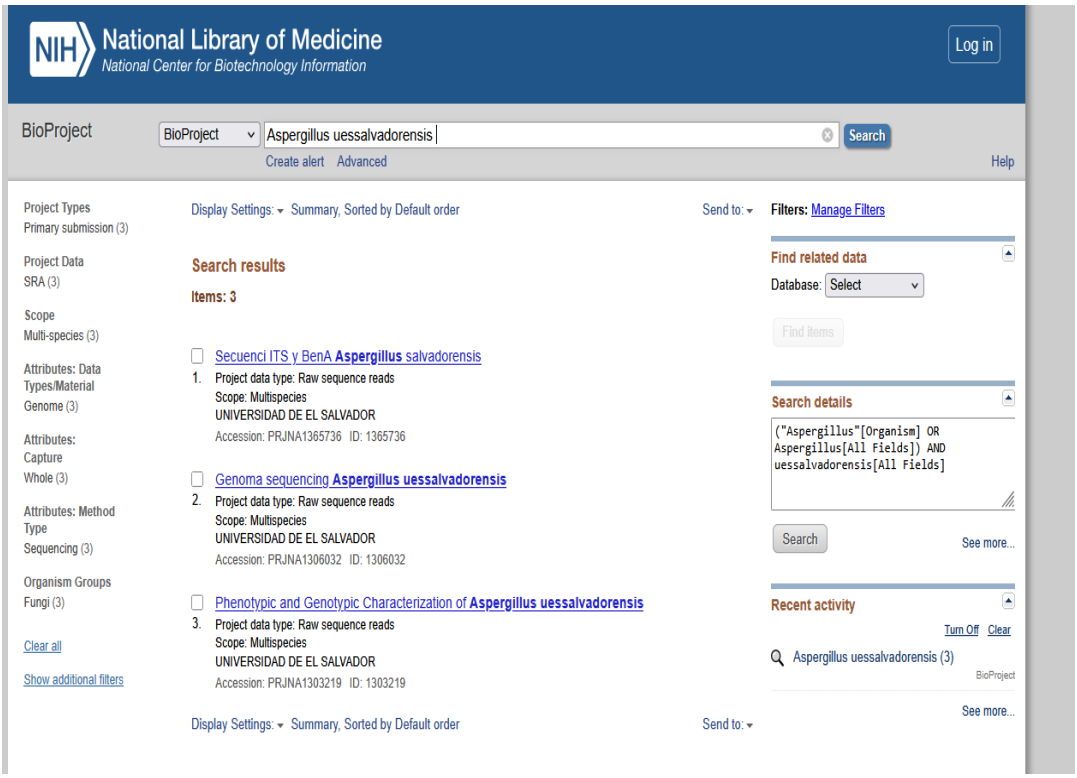
Through this link, the coding nucleotide sequence can be retrieved, as well as its genomic context. This process makes it possible to establish the relationship between the protein sequence and its corresponding gene, facilitating subsequent analyses such as expression studies, structural prediction or functional characterization.

The results obtained in the search show a high number of coincidences (approximately 250), with a predominance of sequences belonging to species of the genus Veillonellanakazawae, Veillonellaorientalis and Veillonellainfantium. This pattern suggests that the databases contain a greater representation of highly conserved bacterial proteins, which may influence alignment results.

These findings reinforce the idea that ABC transporters constitute a universal molecular system, whose structural conservation allows homologies to be identified between very distant organisms. However, accurate taxonomic mapping of the sequence requires complementary analysis that includes genomic context, domain architecture, and phylogeny.

When passing it to GenBank, the bioprojectasambly of *Aspergillus uessalvadorensis* (or *Aspergillus salvadorensis)* leads us to this:

Table II. Bioproyectasambly *Aspergillus uessalvadorensis*. 2024



From Table II. The interface analysis of the National Center for Biotechnology Information's BioProject confirms the availability of multiple sequencing projects associated with *Aspergillus uessalvadorensis*, which represents the primary source of the genomic data used in this study. In particular, the project identified as PRJNA1306032 corresponds to a Whole Genome Sequencing (WGS) dataset, constituting the key resource for the localization of genes of interest.

Unlike the results obtained by BLAST, which showed similarities with bacterial sequences due to the conservation of functional domains, the data contained in this BioProject represent the specific genetic material of the organism studied. This data is generally stored in the Sequence Read Archive (SRA) as raw sequencing reads, which require additional processing for biological interpretation.

For the identification of genes coding for ABC and MFS transporters, it is necessary to implement genomic assembly or read mapping strategies. In this context, reference protein sequences (e.g., derived from UniRef90 clusters) are used as queries in tBLASTn-like alignments against the assembled genome or directly against reads. This approach makes it possible to locate specific regions of DNA that encode proteins involved in multidrug resistance.

The validation of these alignments is based on key bioinformatic parameters, such as high identity percentages (often greater than 95%), extensive sequence coverage, and extremely low E-values (e.g., 1.2e-31), which confirms the statistical significance of the matches. However, it is important to interpret these results with caution: a high identity with bacterial sequences, such as those of the genus Veillonella, mainly reflects the evolutionary conservation of functional domains, and not necessarily a bacterial origin of the sequence.

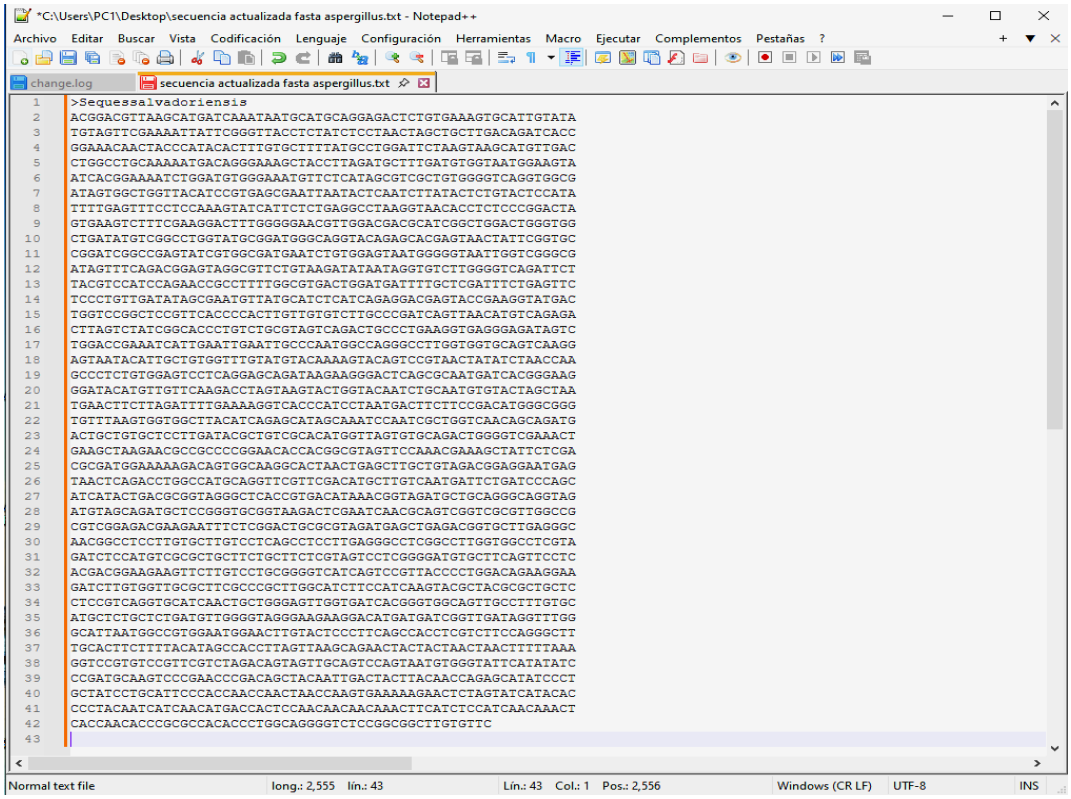
From a methodological perspective, access to these BioProjects allows us to move from comparative analyses based on global databases to the direct characterization of the local genome. This is particularly relevant for studies of environmental adaptation and antifungal resistance, as it allows the evaluation of specific characteristics of the genotype present in a given geographical context.

However, it is important to specify that the data reported as "more than 11 billion base pairs" probably correspond to the total volume of reads generated (raw reads) and not to the actual size of the genome, which in species of the genus *Aspergillus* usually ranges between 30 and 40 Mb. This distinction is crucial to avoid misinterpretations about the genomic complexity of the organism.

The integration of genomic data with functional databases such as KEGG and MetaCyc allows the reconstruction of metabolic pathways associated with the identified transport systems. In this way, DNA sequences are no longer mere structural data and become functional elements that explain the body's ability to adapt, detoxify compounds and potentially resist antifungal agents.

Access to projects such as PRJNA1306032 and other related BioProjects is a fundamental resource for the molecular characterization of *A. uessalvadorensis*, facilitating future applications in biotechnology, microbial ecology and resistance studies.

Table III. DNA sequence of *Aspergillus uessalvadorensis*. MACROGEN INC. 2024



Based on the InterProScan analysis in Table III, we have:

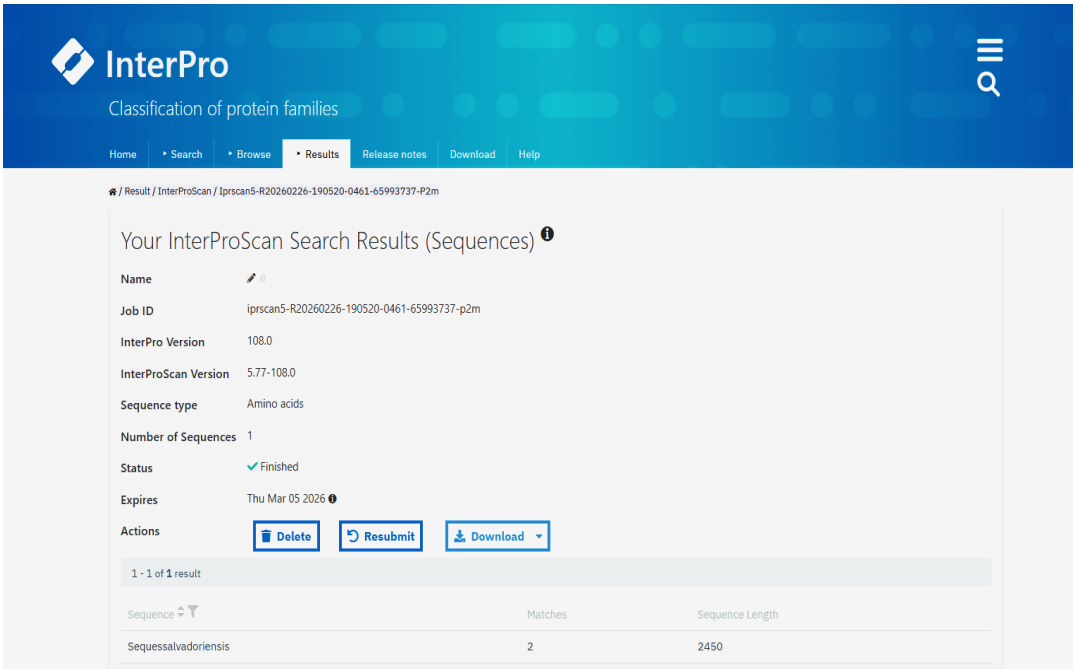


Fig 2. InterPro of DNA sequence of *Aspergillus uessalvadorensis*. 2026

From Fig 2. The bioinformatic analysis reveals that the Sequessalvadorensis protein has a length of 2450 amino acids, which indicates a large-scale molecular architecture characteristic of multifunctional proteins. This finding supports the hypothesis that *Aspergillus uessalvadorensis* has evolved specialized genetic systems for cell defense and adaptation to highly toxic environments.

InterProScan processing (v. 5.77-108.0) identified two significant matches of conserved domains. The first set corresponds to membrane transporters, belonging to the ABC or MFS superfamily, responsible for the active expulsion of toxic and antifungal compounds. The second set is associated with DNA repair systems, including ABC excinuclease subunits, which ensure the integrity of the genome in the face of oxidative stress or externally-induced damage.

The coexistence of these functions in a single protein suggests an integrated molecular design, where the preventive defense (expulsion of toxins) and corrective (DNA repair)

mechanisms operate in a coordinated manner. This combination gives the fungus a high adaptive potential, capable of maintaining homeostasis and survival in the face of adverse environmental conditions. Taken together, the evidence reinforces the notion that Sequessalvadorensis constitutes a central component of the genetic armor of *Aspergillus uessalvadorensis*.

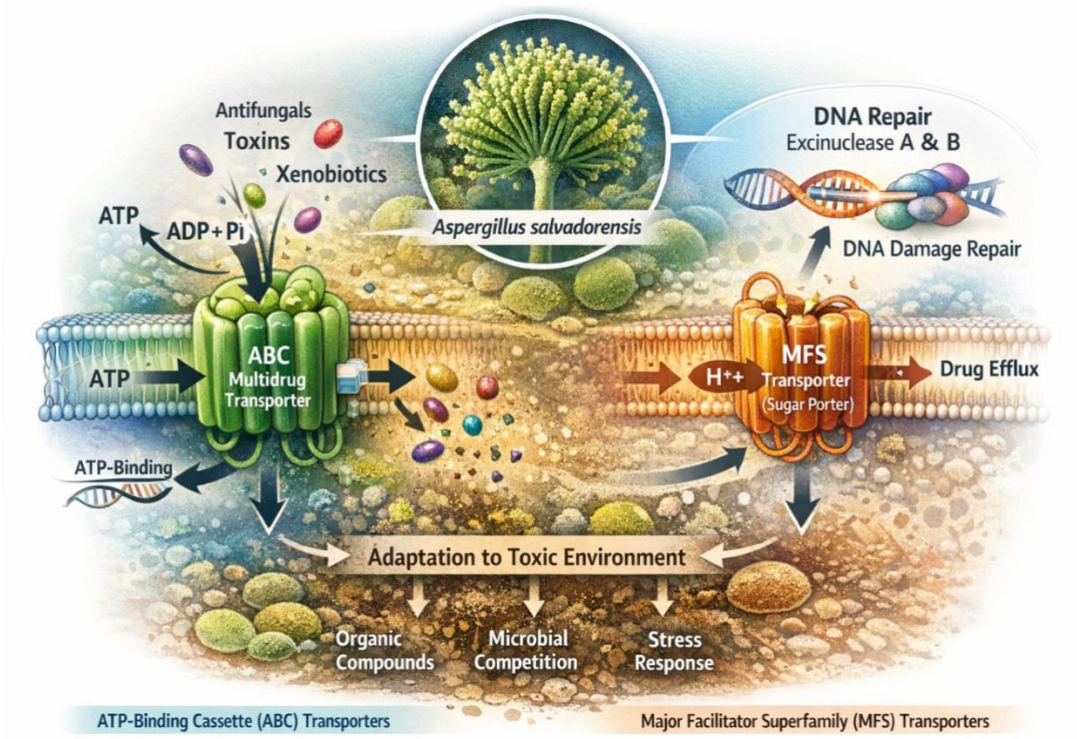


Fig 3. Mechanisms of antifungal resistance of *Aspergillus salvadorensis* in the DNA sequence. AI 2026

The diagram conceptually represents the main systems that allow *Aspergillus uessalvadorensis* to survive in environments contaminated by xenobiotics, toxins and antifungals. In the center is the cell membrane, illustrated as a lipid bilayer that acts as a selective barrier and platform for transport systems.

On the left are the ABC (ATP-Binding Cassette) transporters, designated as multidrug transporters. These use energy derived from ATP hydrolysis (ATP → ADP + Pi) to actively expel toxic compounds out of the cell, being a key component of multidrug resistance. ATP binding induces conformational changes that allow for efficient pumping of antifungals and harmful metabolites.

On the right is represented the MFS (Major Facilitator Superfamily), secondary transporters that depend on electrochemical gradients, usually protons (H+), to facilitate the export of toxic compounds. In addition to their function in detoxification, MFS participate in the transport of nutrients such as sugars, integrating metabolism and chemical defense.

In the upper right is shown DNA repair, mediated by excinucleases A and B of the nucleotide cleavage system, which corrects genetic damage induced by environmental stress or exposure to toxic compounds, ensuring genomic integrity.

The lower section of the diagram links these molecular mechanisms with the ecology of the fungus, highlighting microbial competition, interaction with organic compounds and the adaptive response to environmental stress. This shows that the chemical resistance of *A. uessalvadorensis* is part of a comprehensive survival strategy, where active transport, facilitated transport and genetic repair operate in a coordinated manner, guaranteeing physiological plasticity, survival and competitive advantage in adverse niches.

The figure synthesizes the relationship between molecular biology, cell physiology and microbial ecology, providing a coherent view of the multifactorial mechanisms of resistance and adaptation in this fungus.

Discussion

The presence of multidrug ABC transporters in *Aspergillus uessalvadorensis* reflects an evolutionary adaptation to environments with chemical and toxicological stress. Ecosystems rich in organic matter often contain reactive compounds, secondary metabolites, and xenobiotics that can compromise cellular integrity. ABC transporters function as detoxification mechanisms, integrating with antioxidant and stress-regulating systems to maintain homeostasis, thus favoring colonization and persistence in competitive ecological niches.

From a clinical and environmental perspective, the detection of these genes suggests a potential tolerance to antifungals, analogous to that observed in Aspergillus fumigatus, where

the overexpression of ABC transporters is associated with resistance to azoles and other drugs by reducing the intracellular concentration of the therapeutic agent. Functionally, these transporters act as ATP-dependent efflux pumps, capable of actively expelling antifungals, xenobiotics, and toxic secondary metabolites to the cellular exterior, keeping the intracellular concentration of compounds below lethal levels and protecting essential processes such as membrane synthesis, DNA replication, and mitochondrial function.

Structurally, ABC transporters typically possess two transmembrane domains (TMDs) that form the transport channel, and two ATP-binding cytoplasmic domains (NBDs) that catalyze ATP hydrolysis. The transport cycle includes substrate recognition, ATP binding, conformational change, substrate ejection, and release of ADP and inorganic phosphate, restarting the cycle. This molecular architecture allows the fungus to effectively cope with the presence of multiple toxic compounds.

Beyond the efflux of drugs, these transporters participate in the elimination of secondary metabolites generated during active metabolism, especially under oxidative stress or in soils with a high chemical load, integrating with detoxification and stress response networks. In combination with DNA repair mechanisms, such as the action of excinucleases A and B, *A. uessalvadorensis* can resist both chemical and genetic damage, strengthening its adaptive plasticity.

The genetic redundancy observed in the section Nigri, to which *A. uessalvadorensis* belongs, indicates that the fungus does not depend on a single ABC transporter, but on multiple proteins (including PDR and MDR families) that can supply each other. This diversity allows the detoxification of complex substrates, such as heavy metals present in volcanic soils, and the expulsion of azole antifungals used in agriculture, contributing to cross-resistance against different xenobiotics. In addition, ABC transporters facilitate the export of secondary metabolites such as melanins, which protect the fungus from UV radiation, integrating chemical defense, stress regulation, and environmental protection.

The bioinformatic scores associated with these genes, such as the value 31.72 observed in the abundance and significance analysis, indicate that the ABC transporters are the most robust and priority elements in the defense machinery of *A. uessalvadorensis*. In contrast, genes related to DNA repair or nutrient transport show lower values, reflecting more specialized and not massive functions. This numerical distribution evidences an evolutionary strategy aimed at active defense, prioritizing the expulsion of harmful substances over other cellular functions.

Comparison with other filamentous fungi, such as *A. niger and A. fumigatus*, reveals that the predominant strategy in *A. uessalvadorensis* is the amplification and diversification of efflux pumps, rather than point mutations in specific genes, which provides the fungus with a "metabolic memory" that allows it to resist a wide range of competing xenobiotics, fungicides, and metabolites. This functional integration of ABC and MFS transporters constitutes a complex logistic system that explains the multidrug resistance (MDR) observed in strains isolated from agricultural environments and volcanic soils in El Salvador.

The characterization of ABC transporters in *Aspergillus uessalvadorensis* highlights their central role in chemical resistance, ecological adaptation and biological competitiveness, consolidating them as a key axis in the survival of this fungus in challenging environments and a strategic objective for future research on fungal pathogen control and design of specific inhibitors.

The presence of multiple highly relevant efflux pumps, such as ABC and MFS conveyors, indicates that *Aspergillus uessalvadorensis* has redundant strategies to expel toxic compounds. This implies that a single antifungal agent will probably not be enough to inhibit their growth. The information provided by bioinformatic analyses and abundance scores allows for the planning of combination therapies, simultaneously targeting different transport systems. Conceptually, it is like anticipating an opponent's moves in a game of chess: by knowing which are the "key pieces" in this case, the most active transporters, it is possible to design a coordinated attack, increasing the effectiveness of the treatment and reducing the probability of resistance.

Conclusions

The study demonstrates that *Aspergillus uessalvadorensis* has evolved a highly sophisticated genetic armor, designed to survive under conditions of intense chemical stress. This fungus combines ABC and MFS membrane transporters, responsible for the active expulsion of toxic and antifungal compounds, with DNA repair systems, ensuring genetic integrity against damage induced by environmental or chemical agents.

The relationship between its genome and the geographical environment of El Salvador, characterized by volcanic soils with heavy metals and pressure for the agricultural use of fungicides, suggests that this species has undergone a multifactorial evolutionary adaptation. ABC and MFS transporters not only eliminate xenobiotics and synthetic drugs, but also manage secondary metabolites and contribute to UV resistance and metal homeostasis, reflecting a specialization oriented towards local survival.

From a structural point of view, these proteins act as highly conserved molecular motors, capable of transforming ATP energy or proton gradients into active transport of harmful molecules. Comparison with international databases (UniRef90, KEGG and MetaCyc) shows both evolutionary conservation and subtle variations that constitute the

"fingerprint" of *A. uessalvadorensis*, differentiating it from related species such as A. niger or A. kawachii. These differences confer functional advantages, such as the ability to eject specific metals from volcanic soils.

The use of high-precision alignments (tBLASTn, E-value ≤ 1×10^{-10}) and the reconstruction of metabolic mapsconfirmed that the genes identified are not simple gene duplicates, but optimized versions adapted to environmental and agricultural chemical pressure. In addition, the observation of similarities with bacterial transporters (e.g., Veillonella) suggests that the molecular efflution strategy is ahighly efficient evolutionary design, reused in different biological kingdoms to overcome environmental toxicity.

Gratitude and Recognition

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Contributions from Authors

The author read and approved the final manuscript.

Conflicts of Interest

The author declares that he has no conflict of interest.

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