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***Correspondence**

Antonio Vásquez
Email:
antonio.vasquez@ues.edu.sv

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Physical, Chemical and Biological Properties of *Aspergillus salvadorensis* discovered at the University of El Salvador

Antonio Vásquez Hidalgo 

¹Professor of Microbiology, School of Medicine, University of El Salvador.

Abstract:

The analysis of the physicochemical and biological properties of *Aspergillus salvadorensis*, obtained through DNA sequencing carried out by MACROGEN Inc. (South Korea, 2024), reveals an organism with a key ecological role and a complex network of metabolic and defense pathways. As a saprophytic decomposer, it efficiently degrades dead organic matter, including leaves, wood, and plant debris, and contributes to the recycling of carbon, nitrogen, and minerals, favoring the formation of humus and the natural improvement of the soil. Its metabolism stands out for its ability to break down polysaccharides through glucanases and cellulases and for sustaining energy processes such as heme synthesis, inositol metabolism, and mitochondrial transport. At the cellular level, it integrates pathways such as apoptosis and cell cycle, essential for regulating cell death programmed to stress, hyphal growth, and conidia production, in addition to using meiosis pathways for the formation of ascospores. Cytoskeleton-associated pathways explain the intracellular transport and polarity that enable directed growth. Against oxidative stress, it deploys a robust defense based on melanin and antioxidant pigments, along with enzymes such as catalases, glutathione peroxidases, peroxiredoxins, and thioredoxins. These act in conjunction with cell wall reinforcement, the MAPK (HOG) pathway, calcineurin, PKC, Yap1, and Atf1 transcription factors, and protective metabolites such as mannitol and trehalose. Additional mechanisms, such as DNA repair, chaperones, and proteasomes, ensure survival under stress. In conclusion, *A. salvadorensis* employs a wide range of enzymes, proteins, and secondary metabolites that ensure its resistance and adaptation in oxidative stress environments.



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INTRODUCTION

The study of filamentous fungi of the genus *Aspergillus* has acquired considerable relevance in recent decades due to their metabolic versatility, their ability to synthesize a wide range of extracellular enzymes, and their potential to produce secondary metabolites of high biological and industrial value (Al-Jobory, 2025; Iqbal *et al.*, 2021). Within this genus, *Aspergillus salvadorensis* has recently established itself as a novel species characterized by molecular tools and advanced morphological analyses, offering a window into metabolic pathways that have not yet been fully explored (Borkovich and Ebbole, 2010; Vásquez, 2025a; Vásquez, 2025b). The initial genetic studies carried out in *A. salvadorensis* have made it possible to recognize a set of genes related to the synthesis of about fourteen enzymes, several of which participate in an essential way in oxidative processes and in the production of dark-colored pigments. The identification of these molecular components indicates that the species has developed physiological strategies that strengthen its ability to cope with adverse environmental conditions, which evidences its adaptive ability and the flexibility of its metabolism (Vásquez, 2025a). Likewise, the examination of metabolic clusters has revealed the presence of gene groups linked to the production of secondary metabolites of remarkable structural diversity and functional potential, attributes that could give them a valuable role within the biotechnological field, similar to what has been documented in other species of the genus *Aspergillus* known for their wide metabolic repertoire and bioactive compounds (Bergmann *et al.*, 2007; Chiang *et al.*, 2009; Zakariyah *et al.*, 2024).

The importance of these secondary metabolites lies in the fact that, within the group of fungi, substances such as alkaloids, terpenes and phenolic pigments have been shown to possess outstanding biological activities, including antioxidant properties, antimicrobial effects and selective cytotoxic capacity. These characteristics have boosted interest in various fields, especially in the pharmaceutical, biomedical, and agricultural industries. In this

sense, the conjunction between a broad enzyme system and a heterogeneous secondary metabolism makes *A. salvadorensis* a promising resource for research aimed at the development of innovative technologies based on fungal microorganisms. *A. salvadorensis* is a recently described filamentous species, identified by next-generation sequencing (NGS) and morphological analysis, whose characterization includes not only its genetic profile, but also its metabolic pathways (Vásquez, 2025b). This micromycete has a remarkable enzymatic repertoire; in particular, coding genes for at least enzymes involved in its physiology and in the synthesis of dark pigments have been detected, suggesting an active role in oxidative and cell signaling processes (Borkovich and Ebbole, 2010; Vásquez, 2025a; Vásquez, 2025b). In addition to these catalytic proteins, *A. salvadorensis* presents genetic clusters responsible for the biosynthesis of secondary metabolites, associated with the production of black pigments in response to oxidative stress (Vásquez, 2025b).

The secondary metabolites not only provide an adaptive function against adverse conditions, but also offer biotechnological potential, given that bioactive compounds derived from *Aspergillus* have been widely exploited in the pharmaceutical, food, and environmental industries. The ability of fungi of the genus *Aspergillus* to secrete hydrolases such as proteases, amylases, xylases, and other enzymes, combined with their ability to produce pigments, alkaloids, terpenes, and other secondary metabolites, has been widely documented and constitutes one of the bases of their biotechnological importance (Patil *et al.*, 2021; Rosas-Vega *et al.*, 2025). Therefore, the study of *A. salvadorensis*, with its particular enzymatic and metabolite profile, not only enriches taxonomic and ecological knowledge but also opens up possibilities for innovative industrial applications. Although significant progress has been made in the taxonomic identification of *A. salvadorensis*, there is still a marked scarcity of information on the functioning of its enzymes, the characteristics and functions of its proteins, and the nature of the secondary metabolites it produces. This lack of data makes

it difficult to adequately assess their potential within the biotechnological field, since the absence of detailed information on their biosynthetic pathways makes it impossible to determine their usefulness in industrial, agricultural, or health contexts. Added to this is the impossibility of accurately comparing it with other species of the same genus, which restricts the understanding of its biological particularities and limits the formulation of well-founded proposals on specific applications of its metabolism.

METHODOLOGY

The physicochemical and biological properties of *Aspergillus salvadorensis*, obtained through DNA sequencing carried out by MACROGEN Inc. (South Korea, 2024) were analyzed. The in-depth study of the enzymes, proteins, and secondary metabolites present in *A. salvadorensis* is essential to understand its biological functioning, its ecological role, and the possibilities it offers as a biotechnological resource. Considering that multiple species of the genus *Aspergillus* have established themselves as important sources of hydrolases, natural pigments, and bioactive compounds of industrial and pharmaceutical interest (Meyer *et al.*, 2011), it is reasonable to suggest that *A. salvadorensis* could present a comparable or even greater potential. Deepening the analysis of this species would allow us to identify molecules with applications in biocatalysis, novel therapies, sustainable processes, and other expanding fields within modern biotechnology. In addition, the detailed characterization of this fungus would contribute to expanding knowledge about the microbial diversity of the environment where it was discovered, which is essential to promote its conservation and encourage responsible strategies for the use of local biological resources. In short, the justification of this work is based both on the need to enrich

scientific knowledge about a little-explored species and on the possibility of generating technological, environmental and economic contributions derived from its unique metabolic capacity (Borkovich and Ebbole, 2010; Meyer *et al.*, 2011; Vásquez, 2025a; Vásquez, 2025b).

RESULTS AND DISCUSSION

The raw dataset provided by Macrogen corresponds to a sample identified as *Aspergillus* (Figure 1). In total, 11,705,895,990 base pairs were obtained during the sequencing process, indicating considerable depth for robust genomic analysis. The total number of readings generated amounts to 77,522,490, corresponding to the sum of the readings from the two ends in a paired-end sequencing scheme.

The content of guanine-cytosine (GC) bases represents 49.7% of the total, while adenine-thymine (AT) bases correspond to 50.3%, showing a balanced genome between both types of base pairs, a common characteristic in fungal species of the genus *Aspergillus*.

Regarding the quality of the sequence, the values of Q20 and Q30 reach 95.1% and 88.3%, respectively. These indicators reflect that more than 95% of the bases have a probability of error of less than 1% (Q20), and almost 90% of the bases have an even greater precision, with an error of less than 0.1% (Q30). Together, these values show that the sequencing is highly faithful and suitable for subsequent analyses such as de novo assembly, genomic annotation, or phylogenetic studies.

In summary, the data show efficient sequencing performance and optimal quality that supports the reliability of subsequent bioinformatics analyses.

Raw Data Statistics

The total number of bases, reads, GC (%), Q20 (%), and Q30 (%) are calculated for the 1 sample(s). For example, in *Aspergillus* sample, 77,522,490 reads are produced, and total read bases are 11.7 Gbp. The GC content (%) is 49.7% and Q30 is 88.3%.

* Raw Data

Sample ID	Total bases (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
Aspergillus	11,705,895,990	77,522,490	49.7	50.3	95.1	88.3

- Sample ID : Sample name.
- Total Bases (bp) : Total number of bases sequenced.
- Total Reads : Total number of reads. For illumina paired-end sequencing, this value refers to the sum of read1 and read2.
- GC (%) : Ratio of GC content.
- AT (%) : Ratio of AT content.
- Q20 (%) : Ratio of bases that have phred quality score of over 20.
- Q30 (%) : Ratio of bases that have phred quality score of over 30.

Fig. 1. Raw data from the *Aspergillus* sequence. Macrogen Inc. 2024.

The figure (2) presents a fragment of DNA represented in a three-dimensional structure, accompanied by the assignment of bases and the sequences derived from the three possible reading frames. This information is especially relevant for *Aspergillus salvadorensis*, a recently identified species. In this context, each translated fragment provides key information to determine coding regions, delimit genes, and understand the metabolic potential of the species.

At the bottom are the translations of frames 1, 2, and 3. Each frame generates a different polypeptide string due to the shift of the read start point. Framework 1, which begins with the TDVHK amino acids, could correspond to an active segment of a protein in *A. salvadorensis*, possibly associated with primary or secondary metabolic pathways typical of the genus. **Frame 2**, whose beginning occurs with RTLSM, represents an alternative reading that could be present in regions of high gene density or in superimposed genes, a common phenomenon in filamentous fungi. On the other hand, **frame 3**, which begins with GRA, shows the early presence of a stop codon, suggesting that this reading probably does not correspond to a functional coding region, although it could

participate in local regulation or be part of a pseudo-ORF.

The complexity observed in the three frames is consistent with the genomic structure of *A. salvadorensis*, whose DNA presents organizational patterns similar to those of other species of the genus, but with particularities that are still being discovered through sequencing and bioinformatic analysis. The three-dimensional representation of the double helix facilitates the understanding of the orientation of the fragment and allows visualization of how the nucleotides are spatially organized, which is useful for interpreting regions with possible transcriptional activity or with structural functions within the genome.

This analysis illustrates how a single fragment of *A. salvadorensis* DNA can give rise to multiple protein products depending on the reading frame used, reflecting the versatility and biological complexity of the species. This variability is particularly important as it is a new species, as it contributes to the identification of genes associated with environmental adaptation mechanisms, metabolite production, enzyme activity, and possible virulence factors or ecological interaction.

Overall, the figure integrates structure, sequence, and translation to offer a detailed view of the coding potential of a fragment of the *Aspergillus salvadorensis* genome, providing

fundamental elements for its future genomic annotation and functional understanding.

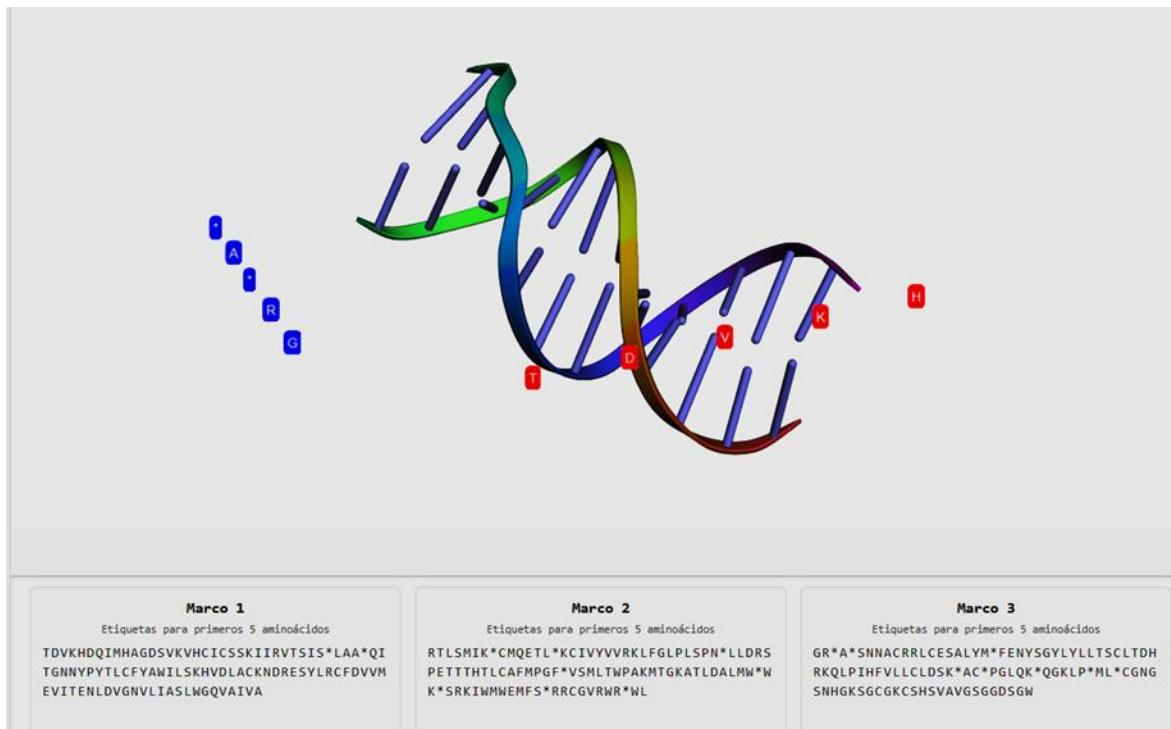


Fig. 2. Frames for reading the DNA sequence of *Aspergillus salvadorensis*. AI. Macrogen 2024.

In General, the functional analysis indicated the presence of pathways linked to both programmed cell death and unprogrammed forms of cell elimination. The elements assigned to apoptosis showed correspondence with conserved eukaryotic mechanisms, including a module similar to that described in insect models, which suggests that the fungus could resort to regulated mycelial remodeling processes. At the same time, the detection of components associated with necroptosis suggests the activation of cellular responses characteristic of severe stress scenarios (Steinberg, 2011). The pathways related to the cell cycle, derived from comparative schemes with *Caulobacter* and yeasts, show a typical organization of the G1, S, G2, and M phases, consistent with the nuclear dynamics of a multinucleated mycelium. To these are added the meiotic pathways, represented by yeast

models and by pathways analogous to *oocyte meiosis*, which include regulators involved in recombination, chromosomal cohesion, and control of nuclear progression, consistent with the ability to form ascospores (Jia *et al.*, 2025; Mansour *et al.*, 2025; Pollack *et al.*, 2009; Shlezinger *et al.*, 2012; Suelmann and Fischer, 2000; Taborda *et al.*, 2008). Examination of individual proteins allowed key metabolic functions to be delineated. An endo- β -glucanase (uniref90_a2r5l7) was associated with the degradation of structural polysaccharides of the substrate, while a TPP transporter (uniref90_a2r5a0) and a ferrochelatase (uniref90_a2r549) were related to respiratory processes and heme synthesis. Likewise, enzymes linked to the inositol pathway (uniref90_a2r561) and regulatory factors of nitrogen assimilation (uniref90_a2r522) pointed to a role in the use of carbon reserves and in the

processing of nitrogenous compounds. Finally, the presence of thioredoxin-type proteins (uniref90_a2r5a2) indicated the existence of antioxidant systems aimed at preserving cellular redox balance. The organization of the cytoskeleton emerged as an integrative component of multiple cellular functions. Pathways of actin cytoskeleton regulation, microtubule assembly, and motor protein activity including myosin's, kinesins, and dynein's reveal processes involved in intracellular transport, polarity, and apical growth of hyphae (Harris, 2001; Ibrahim *et al.*, 2023; Nebenführ and Dixit, 2018; Oakley and Peñalva, 2025; Shlezinger *et al.*, 2017; Takeshita and Fischer, 2019; Wu *et al.*, 1998).

Aspergillus salvadorensis is considered an environmental fungus with a fundamental ecological role (Table 1) due to its ability as a saprophytic decomposer, responsible for degrading dead organic matter such as leaves, wood, and plant remains. This activity allows the release of carbon, nitrogen, minerals, and other essential elements that feed the nutrient cycle and sustain the biogeochemical dynamics of the ecosystem. Its intervention in decomposition favours the formation of humus and, with it, the improvement of the structure, fertility, and quality

of the soil, increasing its water retention capacity and promoting healthy microbiota. Its carbohydrate metabolism is highly efficient, producing enzymes such as glucanases and cellulases capable of breaking down complex polysaccharides such as β -glucans and cellulose, allowing it to tap into plant-based carbon sources. At the energy level, it depends on essential pathways such as heme synthesis, inositol metabolism, and mitochondrial transport, which allow it to adapt to various environments and maintain stable metabolic activity. As for the cellular response, it has antioxidant mechanisms based on thioredoxins and other protective proteins, which allow it to manage oxidative stress and preserve the integrity of its structures. This fungus is not considered a significant pathogen, as it has not been associated with diseases in humans, animals, or plants, and its presence is usually related to positive functions in the environment. Overall, *A. salvadorensis* represents a beneficial organism that contributes to ecological balance, biological recycling, and the maintenance of healthy and productive soils (Oakley and Peñalva, 2025; Riquelme, 2013; Shlezinger *et al.*, 2012; Shlezinger *et al.*, 2017; Steinberg, 2007; Takeshita *et al.*, 2014; Tanaka *et al.*, 2022; Vásquez, 2025a; Vásquez, 2025b).

Table 1. Functions of the fungus *Aspergillus salvadorensis*. MACROGEN Report. 2025

Categoría	Función	Descripción
Ecological role	Decomposer (saprophyte)	It decomposes dead organic matter such as leaves, wood, and plant debris.
Nutrient Cycling	Carbon, nitrogen, and mineral recycling	It releases nutrients into the soil after the degradation of organic compounds.
Impact on the ground	Improves and maintains soil quality	Contributes to the formation of humus and soil fertility.
Carbohydrate metabolism	Degradation of complex polysaccharides	It produces enzymes such as glucanases and cellulases that break down β -glucans and cellulose.
Energy metabolism	Essential metabolic processes	It includes pathways such as heme synthesis, inositol metabolism, and mitochondrial transport.
Cellular response	Oxidative stress management	It has thioredoxin-type proteins that protect against oxidative stress.
Pathogenicity	Not a significant pathogen	It is not known to cause disease in humans or plants.
General importance	Beneficial environmental organization	Contributes to ecological balance and biological recycling.

Table (2) shows that the pathways grouped under cellular motility do not refer to the movement of the whole organism, but to the internal organization that allows the directed

growth of the hyphae. The cytoskeleton pathways, although in the database appear associated with muscle cells or flagella, in *Aspergillus* represent the organization of actin

and microtubules that allow vesicles, organelles, and material to be transported to form the cell wall at the growth tip. Motor proteins, such as kinesins and dyneins, facilitate this internal transport and the correct division of nuclei. Finally, the regulation of the actin cytoskeleton action is essential to maintain the polarity of the hyphae, control the growth of mycelium, and

respond to different types of environmental stresses. Together, all these pathways describe processes that allow *A. salvadorensis* to grow, reproduce, maintain its structure, respond to adverse conditions, and organize its internal cellular activity (Ameisen, 2002; Carmona-Gutierrez *et al.*, 2018; Takeshita *et al.*, 2014; Wu *et al.*, 1998).

Table 2. Secondary metabolic functions of *Aspergillus salvadorensis*. MACROGEN Report. 2025

Función	Relación con las proteínas estudiadas	Descripción breve
Carbohydrate Breakdown	Endo-β-glucanasas (uniref90_a2r517)	Rompe β-glucanos of the cell wall and complex polysaccharides of the environment.
Energy metabolism and cofactors	Carrier tpp (uniref90_a2r5a0), ferroquelatasa (uniref90_a2r549)	It participates in mitochondrial metabolism, the Krebs cycle, and heme biosynthesis.
Lipid and nitrogen metabolism	Inositol Enzymes (uniref90_a2r561), reguladores de nitrógeno (uniref90_a2r522)	It processes nitrogenous compounds and reserves carbohydrates for energy and cell synthesis.
Cell protection / oxidative stress	Type proteins (uniref90_a2r5a2)	Maintains redox balance and protects against oxidative damage.
General ecological role	All of the above	Saprophytic decomposer, recycles nutrients, contributes to the balance of the soil and ecosystem.

A. salvadorensis has multiple cellular pathways that act in an integrated manner to sustain growth, differentiation, and environmental adaptation (Table 3). The elements associated with apoptosis play a key role in mycelium homeostasis, as they promote the orderly elimination of deteriorated or dysfunctional cells and contribute to the remodeling of the hyphal network during development. The presence of a module similar to the *fly apoptosis* model suggests that the fungus retains central principles of activation and execution of programmed cell death, adjusted to its fungal architecture. In addition, the identification of components linked to necroptosis points to the existence of mechanisms of unprogrammed cell death that are activated under conditions of intense stress, allowing the removal of severely affected mycelial regions and participating in defense responses (Aguirre *et al.*, 2005; Ameisen, 2002; Horio and Oakley, 2005; Jangid *et al.*, 2024; Rangel, 2011; Wu *et al.*, 1998). The pathways associated with the cell cycle represent an essential regulatory axis for hyphal expansion and nuclear division, processes that support both vegetative growth and conidia production. Comparative models with

Caulobacter and yeast allow us to interpret conserved steps—including DNA replication, chromosomal segregation, and cytokinesis and provide a framework for understanding how *A. salvadorensis* coordinates the G1, S, G2, and M phases in a multinucleate system. These routes favor the analysis of the spatial organization of divisions into compartmentalized hyphae and nuclear synchronization during sporulation (Carmona-Gutierrez *et al.*, 2018; Semighini and Harris, 2010; Takeshita and Fischer, 2019). From a metabolic perspective, the route noted as *alcoholism* groups together the processes that allow ethanol and other alcohols to be used as alternative sources of carbon. The induction of alcohol and aldehyde dehydrogenases makes it possible to channel these substrates into central pathways of energy metabolism, which gives the body physiological flexibility in environments where the availability of sugars is limited. These meiosis-associated pathways, derived from yeast models, indicate that *A. salvadorensis* possesses the machinery necessary to carry out sexual reproduction and the formation of ascospores through genetic recombination and chromosomal reduction. This set is complemented by components analogous

to those described in *oocyte meiosis*, which include kinases, cohesins, and nuclear checkpoints, and which reflect regulatory principles widely conserved in eukaryotes. The combination of these pathways offers an integrative view of the mechanisms that govern nuclear dynamics during the asexual phase of

the life cycle (Berepiki *et al.*, 2011; Carmona-Gutierrez *et al.*, 2018; Durand and Ramsey, 2023; Ibrahim *et al.*, 2023; Semighini and Harris, 2010; Steinberg, 2011; Takeshita and Fischer, 2019; Vásquez, 2025a).

Table 3. Functions of cell pathways in *Aspergillus salvadorensis*. MACROGEN Report. 2025

Ruta	Función en <i>aspergillus salvadorensis</i>
Apoptosis	It controls programmed cell death, eliminates damaged cells, and regulates the development of mycelium.
Apoptosis – fly	It represents the same programmed cell death pathway, based on a different model, but with the same function in fungi.
Cell cycle	Regulates nuclear division, hyphae growth, and conidia formation.
Cell cycle – Caulobacter	It describes conserved cell cycle steps applied to hyphae growth and division.
Cell cycle – yeast	Based on the yeast model, but controls the same phases of the cell cycle in <i>Aspergillus</i> .
Alcoholism	Degradates ethanol as a source of carbon and energy
Meiosis – yeast	It controls sexual reproduction and the formation of ascospores during the meiotic phase.
Necroptosis	It describes mechanisms of unprogrammed cell death that can be activated under severe stress.
Oocyte meiosis	Although derived from an animal model, it represents the general mechanisms of meiosis used in the sexual reproduction of the fungus.
Cytoskeleton in muscle cells	It indicates the organization of the cytoskeleton that allows intracellular transport and growth at the tip of the hyphae.
Flagellar assembly	Although <i>Aspergillus</i> does not possess flagella, this pathway includes genes involved in microtubule organization and cell transport.
Motor proteins	It controls the movement of vesicles, organelles, and nuclei within the hyphae using myosins, kinesins, and dyneins.
Regulation of the actin cytoskeleton	It regulates cell polarity, hyphal growth, and the distribution of vesicles necessary to form cell walls.

The cytoskeleton is another fundamental axis of cellular organization. Although some of the identified pathways were originally described in animal cells, their function in *A. salvadorensis* is associated with the regulation of actin and microtubule architecture and dynamics, essential for intracellular transport, polarity establishment, and apical growth of hyphae. The actin cytoskeleton regulatory pathway is involved in vesicle redistribution required for cell wall synthesis and polarized growth orientation. In addition, the flagellar assembly pathway, despite the fact that the genus *Aspergillus* lacks flagellate structures, integrates factors involved in the nucleation and stabilization of microtubules, critical components for cytoplasmic trafficking. These functions are coordinated with the activity of myosins, kinesins, and dyneins, described in the motor protein pathway, responsible for the transport of

vesicles, organelles, and nuclei along the hyphae (Durand and Ramsey, 2023; Harris, 2001; Jacobsen, 2000; Takeshita and Fischer, 2019; Vásquez, 2025b). Taken together, the identification of these pathways reveals a complex functional network that explains the organism's ability to sustain its growth, respond to environmental variations, and complete its life cycle. This integrative overview provides a solid basis for understanding the cellular and molecular organization of *A. salvadorensis*. Exposure to oxidative stress activates a set of a wide range of mechanisms aimed at limiting the impact of reactive oxygen species (ROS) generated by free radicals, toxic compounds, UV radiation, transition metals, and ethanol or host immune responses. These mechanisms protect essential components of the cell, including proteins, membranes, DNA, and the cell wall. Among them, melanin is a central defensive

element. In *Aspergillus*, this pigment works as a powerful antioxidant agent capable of neutralizing hydrogen peroxide, superoxide radical, and hydroxyl radical, as well as strengthening the cell wall by forming a protective barrier against physical and chemical damage. Melanin also attenuates the penetration of UV radiation and reduces the entry of metals involved in Fenton reactions, contributing to survival in harsh environments and during interaction with host defensive mechanisms. Its ability to scavenge free radicals efficiently limits intracellular damage associated with oxidative stress (Harris, 2001; Horio and Oakley, 2005; Ibrahim *et al.*, 2023; Nji *et al.*, 2023; Steinberg, 2007; Tanaka *et al.*, 2022). At the enzymatic level, *A. salvadorensis* displays a characteristic antioxidant repertoire of filamentous fungi. Among the most relevant enzymes are superoxide dismutases, which convert the superoxide radical into hydrogen peroxide; catalases, responsible for breaking down this peroxide into water and oxygen; and the glutathione-dependent system, made up of glutathione peroxidases and reductases that are involved in the detoxification of oxidants. In addition, proteins from the thioredoxin and peroxiredoxin pathways are added, which preserve the functionality of cellular proteins exposed to oxidizing conditions. The expression of these enzymes usually increases when the fungus detects signs of stress, reinforcing its resilience (Ibrahim *et al.*, 2023; Oakley and Peñalva, 2025). As a complementary response, *A. salvadorensis* strengthens the structure of your cell wall. Under oxidative stress, it increases the synthesis of chitin and β -glucans, reorganizes the actin cytoskeleton to facilitate the repair of structural damage, and thickens the wall, increasing its resistance to ROS and toxic compounds. The additional deposition of melanin in this matrix contributes to improved protection against aggressive environmental conditions. *A. salvadorensis* tackles oxidative stress through a combination of defenses: it produces melanin that acts as an antioxidant and physical barrier; it activates enzymes that eliminate free radicals; it thickens and protects its cell wall; it uses compatible metabolites such as mannitol or trehalose; it repairs molecular damage and reorganizes its physiology by

signaling pathways such as *mapk* and *yap1*. Together, these responses allow the fungus to survive in harsh environments and maintain its growth even under severe stress conditions (Aguirre *et al.*, 2005; Shlezinger *et al.*, 2017).

In *A. salvadorensis*, a set of cellular pathways operates in an integrated manner to sustain growth, differentiation, and adaptation to the environment (Table 4). The apoptosis pathway plays an essential role in maintaining mycelial homeostasis, as it allows for the regulated removal of aged, damaged, or functionally compromised cells, contributing to structural remodeling during development. The presence of a module similar to that described in the *fly apoptosis* model suggests that the fungus retains basic principles of signaling and execution of programmed cell death observed in distant eukaryotes, adapted to their fungal organization. In a complementary way, the components associated with necroptosis indicate the existence of mechanisms of unprogrammed cell death that are activated under conditions of intense stress, allowing the removal of irreversibly affected mycelial regions and participating in defensive responses (Aguirre *et al.*, 2005; Ibrahim *et al.*, 2023; Nji *et al.*, 2023; Tanaka *et al.*, 2022).

The pathways related to the cell cycle constitute a central axis of regulation of hyphal growth and nuclear division, essential processes for vegetative expansion and the formation of conidia. Comparative models with *Caulobacter* and yeast allow the identification of conserved stages of DNA replication, chromosomal segregation, and cytokinesis, providing a conceptual framework to interpret how *A. salvadorensis* organizes and coordinates the G1, S, G2, and M phases within a multinucleated mycelium. These pathways are particularly useful for analyzing the spatial distribution of divisions in compartmentalized hyphae and the timing of nuclear events during sporulation. On the metabolic level, the pathway noted as *alcoholism* groups together the processes that allow the use of ethanol and other alcohols as alternative sources of carbon. The induction of alcohol and aldehyde dehydrogenases makes it possible to

incorporate these compounds into central pathways of energy metabolism, providing the body with remarkable physiological versatility in environments with reduced availability of simple

sugars (Oakley and Peñalva, 2025; Semighini and Harris, 2010; Takeshita and Fischer, 2019).

Table 4. *Aspergillus salvadorensis* response to oxidative stress. MACROGEN Report. 2025

Mechanism	Role in the response to oxidative stress	
Melanin production	It acts as an antioxidant, neutralizes ros, protects the cell wall, reduces UV light damage, and limits the entry of metals that generate free radicals. Induces pigmentation for fungal dye.	
Superoxide dismutases (sod)	Convert the superoxide radical into less toxic hydrogen peroxide.	
Catalasas	They remove hydrogen peroxide by transforming it into water and oxygen.	
Glutathione peroxidases and reductases	Neutralize oxidants using glutathione and maintain redox balance.	
Peroxirredoxins and thioredoxins	Protect cellular proteins from oxidative damage and repair sulfhydryl groups.	
Cell wall strengthening	Increased chitin and β -glucans; Strengthens resistance to rust and toxins.	
Reorganización del citoesqueleto (actina)	Repairs wall damage and keeps polarized growth under stress.	
Ruta mapk (hog pathway)	Detects oxidative stress and activates defense and repair genes.	
yap1/atf1 transcription factors	They activate the expression of antioxidant and detoxification genes.	
Vía de calcineurina y pkc	Maintain membrane and cell wall integrity under oxidative conditions.	
Manitol y trehalosa	They act as antioxidant metabolites that sequester free radicals.	
Pigments and secondary metabolites	They work as additional natural antioxidants.	
DNA repair	Corrects oxidative damage in genetic material.	
Chaperonas (hsp)	They fold proteins damaged by reactive oxygen.	
Proteasoma	It degrades oxidized proteins that cannot be repaired.	
Pigment producer	When oxidized, 14 enzymes and secondary metabolites were found in the production of black pigments produced by the fungus through oxidative stress.	

The pathways associated with meiosis, derived from yeast models, indicate that *A. salvadorensis* has the necessary machinery to carry out asexual reproduction and the formation of ascospores through genetic recombination and chromosomal reduction. These processes are complemented by components analogous to those described in animal systems, such as *oocyte meiosis*, which illustrate widely conserved regulatory mechanisms, including the involvement of kinases, cohesins, and checkpoints that also play essential roles in the meiotic progression of fungi. The integration of these models allows for a more complete understanding of the nuclear dynamics that characterize the sexual phases of the life cycle. The cytoskeleton represents another key functional axis in the cellular organization of *A. salvadorensis*. Although some routes are based on analyses carried out on muscle cells or mobile organisms, their relevance in this fungus

lies in the regulation of actin and microtubule dynamics, essential for intracellular transport, polarity establishment, and apical growth of hyphae. The actin cytoskeleton regulatory pathway is involved in the distribution of vesicles involved in the synthesis and remodeling of the cell wall, while the flagellar assembly pathway, despite the absence of flagella in *Aspergillus*, incorporates factors related to the nucleation and stabilization of microtubules, essential elements for cytoplasmic transport. These functions are integrated with molecular motor activity, described in the motor protein pathway, which includes myosins, kinesins, and dyneins responsible for the direct movement of vesicles, organelles, and nuclei along the hyphae, ensuring the continuous supply of materials towards the growth tip (Oakley and Peñalva, 2025). Together, these pathways reveal the functional complexity of *A. salvadorensis* and provide an integrative picture of the cellular and

molecular processes that underpin its development, adaptability, and life cycle compliance. Melanin DHN is a pigment derived from the polyketide pathway, which are chemical precursor. The enzyme PksP (polyketide synthase) is responsible for initiating the synthesis of the main precursor (a heptacetide). This pigment is deposited on the cell wall of the spores, essentially acting as a protective shield for the survival of the fungus. The production of melanin is not constant but is induced (active) when the fungus is faced with stressful conditions, especially oxidative stress (caused by the accumulation of reactive oxygen species, such as free radicals). The accumulation of free radicals acts as a crucial cellular signal, activating genes that allow the fungus to build the melanin defense machinery to survive.

Although the enzyme NADH-quinone oxidoreductase (NDH), or Complex I, does not produce melanin directly, it is essential for its synthesis: NDH is essential because it generates energy (ATP) and maintains the necessary metabolic balance. Without the energy and homeostasis provided by NDH, the enzymatic machinery of DHN-melanin (such as PksP) would not be able to function or generate pigmented defenses, which could lead to the death of the fungus. In short, stress activates the melanin machinery, but NDH provides it with the fuel to function (Harris, 2001; Horio and Oakley, 2005; Ibrahim *et al.*, 2023; Jacobsen, 2000; Nji *et al.*, 2023; Tanaka *et al.*, 2022; Vásquez, 2025a; Vásquez, 2025b).

The study of the physicochemical and biological characteristics of *A. salvadorensis*, based on DNA sequencing conducted by MACROGEN Inc. (South Korea, 2024), shows that this fungus plays an important ecological role and possesses an intricate system of metabolic and defense pathways (Figure 3). As a saprophytic

decomposer, it breaks down dead organic material, including leaves, wood, and other plant residues, facilitating the recycling of carbon, nitrogen, and minerals, and helping build humus that naturally enriches soil quality. Its metabolic capabilities include the degradation of polysaccharides through β -glucanase and cellulase enzymes, along with the operation of key energy-related processes such as heme synthesis, inositol metabolism, and mitochondrial transport functions.

At the cellular level, *A. salvadorensis* engages pathways that govern apoptosis and the cell cycle, which are essential for managing programmed cell death under stress, promoting hyphal growth, and enabling conidia formation; it also relies on meiotic mechanisms to produce ascospores. Pathways linked to the cytoskeleton support intracellular transport and polarity, both of which drive directional growth.

When exposed to oxidative stress, the fungus activates a strong protective response that includes melanin and other antioxidant pigments, supported by enzymes like catalases, glutathione peroxidases, peroxiredoxins, and thioredoxins. These components act together with cell-wall strengthening mechanisms, the HOG (MAPK) stress pathway, calcineurin signaling, PKC, and transcription factors such as Yap1 and Atf1. Protective metabolites, including mannitol and trehalose, further enhance its resilience. Additional systems, such as DNA repair pathways, molecular chaperones, and the proteasome, preserve cellular integrity under harsh conditions. Overall, *A. salvadorensis* relies on a broad array of enzymes, proteins, and secondary metabolites that support its adaptability, stress tolerance, and ecological importance in environments exposed to oxidative or structural challenges.

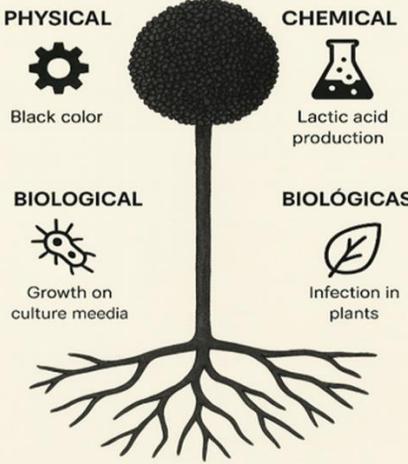
<p>Physical:</p> <ul style="list-style-type: none"> Black coloration associated with spores and reproductive structures. Production of melanin that provides pigmentation and protection. Typical morphology of <i>Aspergillus</i>: septate hyphae and erect conidiophores. <p>Biological:</p> <ul style="list-style-type: none"> Efficient saprophyte: decomposition of leaves, wood, and plant remains. Contribution to the recycling of carbon, nitrogen, and minerals in the soil Activation of apoptosis and cell cycle pathways for growth and response to stress Formation of conidia and ascospores (asexual and sexual reproduction). Robust defenses against oxidative stress: catalases, glutathione peroxidases, peroxiredoxins, thioredoxins. 	<p>PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF <i>Aspergillus salvadorensis</i></p>  <table border="1"> <thead> <tr> <th>PHYSICAL</th> <th>CHEMICAL</th> </tr> </thead> <tbody> <tr> <td> Black color</td> <td> Lactic acid production</td> </tr> <tr> <th>BIOLOGICAL</th> <th>BIOLÓGICAS</th> </tr> <tr> <td> Growth on culture media</td> <td> Infection in plants</td> </tr> </tbody> </table>	PHYSICAL	CHEMICAL	 Black color	 Lactic acid production	BIOLOGICAL	BIOLÓGICAS	 Growth on culture media	 Infection in plants	<p>Chemical:</p> <p>Ability to degrade polysaccharides by glucanases and cellulases.</p> <ul style="list-style-type: none"> Involvement in key metabolic pathways: heme synthesis, inositol metabolism, and mitochondrial transport. Production of protective metabolites such as mannitol and trehalose Production of antioxidant pigments and other secondary metabolites. <p>Biological:</p> <p>Use of MAPK (HOG), calcineurin, PKC, and Yap1 and Atf1 transcription factors</p> <ul style="list-style-type: none"> Cell wall reinforcement and polarity mechanisms for targeted growth. DNA repair systems, chaperones, and proteasome for cell stability.
PHYSICAL	CHEMICAL									
 Black color	 Lactic acid production									
BIOLOGICAL	BIOLÓGICAS									
 Growth on culture media	 Infection in plants									

Fig.3. Physical, chemical, and biological properties of *Aspergillus salvadorensis*. Al. MACROGEN. 2025

The results obtained indicate that *A. salvadorensis* shares with other filamentous fungi widely conserved regulatory principles, although it presents functional particularities that probably reflect its adaptation to a specific ecological niche. The pathways associated with apoptosis and necroptosis reveal the coexistence of complementary mechanisms of cellular control: on the one hand, the programmed remodeling of the mycelium through regulated cell death, and on the other, the rapid elimination of irreversibly damaged regions under conditions of severe stress. Similarly, the identification of complete meiotic pathways suggests the organism's ability to alternate between asexual and sexual reproduction, a strategy that contributes both to the generation of genetic variability and to adaptation to changing environments (Jangid *et al.*, 2024; Shlezinger *et al.*, 2012; Wu *et al.*, 1998). The set of metabolic pathways detected supports the physiological versatility of the fungus to take advantage of substrates present in environments rich in organic matter. The activity of endo- β -glucanases confirms its potential to degrade structural polysaccharides from plant biomass, while the amplitude of its energy metabolism, together with the integration

of heme, inositol, and nitrogen pathways, points to a robust and flexible metabolic network. The presence of antioxidant proteins, such as thioredoxins, also reflects an adaptation to fluctuations in the redox state, characteristics of saprophytic niches exposed to environmental variability (Shlezinger *et al.*, 2017). The complex organization of the cytoskeleton and the involvement of molecular motors suggest a highly specialized intracellular transport system, consistent with the polarized growth typical of filamentous fungi. These processes are essential for the direct distribution of vesicles, the synthesis and remodeling of the cell wall, and the maintenance of apical polarity, indispensable features for the architecture and dynamics of mycelium. The characterization of its cellular and metabolic pathways is key to understanding how this organism sustains its growth, coordinates mycelium differentiation, and responds to environmental variations. Processes such as regulated cell death, nuclear cycle control, meiosis, and cytoskeleton dynamics are essential to maintain the structural integrity of filamentous fungi. Similarly, enzymatic degradation of polysaccharides, energy generation, nitrogen assimilation, and antioxidant responses determine their ability to

exploit complex substrates and thrive in environments rich in organic matter (Carmona-Gutierrez *et al.*, 2018; Vásquez, 2025b; Vásquez, 2025c). In this work, functional pathways and representative proteins identified in *A. salvadorensis* are analyzed to outline a general framework of its physiology and ecological potential. The analysis of regulatory pathways indicated the presence of components associated with both programmed and stress-induced cell death. The proteins assigned to the apoptosis pathway showed similarity with well-characterized eukaryotic mechanisms, including a version comparable to the fly apoptosis model, suggesting the existence of controlled mycelial remodeling processes. At the same time, elements compatible with necroptosis were detected, which could participate in the elimination of severely damaged regions of the mycelium. The cell cycle pathways revealed a set of regulators homologous to those described in *Caulobacter* and yeast, encompassing G1, S, G2, and M phases. These signals are consistent with a multinucleated nuclear system and with the need to coordinate divisions into compartmentalized hyphae. Likewise, components of the meiotic machinery were identified, both derived from yeast models and from oocyte meiosis pathways, which include factors involved in chromosomal cohesion, recombination, and control points. Analysis of annotated proteins showed key metabolic functions. An endo- β -glucanase (uniref90_a2r5l7) was associated with the hydrolysis of structural polysaccharides. A TPP conveyor (uniref90_a2r5a0) and a ferrochelatase (uniref90_a2r549) were associated with mitochondrial processes and heme synthesis. Enzymes linked to inositol metabolism (uniref90_a2r561) and nitrogen assimilation regulators (uniref90_a2r522) indicated versatility for processing carbon and nitrogenous compounds. In addition, thioredoxin (uniref90_a2r5a2) proteins suggest the existence of active defense systems against oxidative stress obtained from the result of the *Aspergillus* sequence in MACROGEN INC., which are similar to other fungi of the genus *Aspergillus*. Finally, pathways related to cytoskeleton organization, actin regulation, microtubule dynamics, and molecular motor

activity (myosins, kinesins, and dyneins) were observed. These processes support intracellular transport, vesicle distribution, and the polarized growth characteristic of hyphae. The results suggest that *A. salvadorensis* shares fundamental elements of the cellular regulation observed in other filamentous fungi, while possessing particularities that could be associated with its saprophytic lifestyle. The apoptosis and necroptosis pathways suggest that this organism maintains complementary strategies to control mycelium quality and respond to severe damage (Steinberg, 2011; Wu *et al.*, 1998). The presence of a complete meiotic repertoire indicates that it retains the ability to reproduce sexually and generate genetic diversity. In metabolic terms, the identification of an endo- β -glucanase and various energy and nitrogen regulators supports a physiological model adapted to the degradation of plant biomass and the exploitation of variable resources. Antioxidant systems, represented by thioredoxins, could be essential to tolerate the fluctuating conditions typical of microhabitats rich in organic matter. The complex organization of the cytoskeleton indicates a refined control of intracellular transport and apical growth, essential processes for the effective colonization of the substrate. The set of pathways and proteins characterized in *Aspergillus salvadorensis* suggests a functional network that supports its degradative capacity, its metabolic plasticity, and its ecological role as a saprophytic organism. The combination of cell death mechanisms, nuclear regulation, versatile metabolism, and a dynamic cytoskeletal architecture provides an integrative basis for understanding their adaptation and their contribution to nutrient recycling in natural environments (Durand and Ramsey, 2023; Oakley and Peñalva, 2025).

CONCLUSION

The repertoire of pathways and proteins identified in *Aspergillus salvadorensis* evidences a highly integrated functional network that supports its growth, its metabolic versatility, and its ecological role as a saprophytic organism.

The articulation of cellular processes that encompass regulated cell death, nuclear cycle control, meiosis, and cytoskeleton dynamics with metabolic capacities aimed at polysaccharide degradation, energy generation, and protection against oxidative stress offers a holistic picture of its physiology. Together, these elements position *A. salvadorensis* as a significant microorganism within soil microbial communities, with a substantial contribution to nutrient recycling and organic matter transformation.

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CONFLICT OF INTEREST

The author declares that he has no conflicts of interest.

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