IKR Publishers

IKR Journal of Agriculture and Biosciences (IKRJAB)
Journal homepage: https://ikrpublishers.com/ikrjab/
Volume-2, Issue-3 (May-June) 2026
OPEN ACCESS
ISSN: 3107-5509 (Online)

Characterization of the Catalase Gene in *Aspergillus salvadorensis* [1] and its Role in Oxidative Stress

Dr. Antonio Vásquez Hidalgo, PhD*
Professor Microbiology, School of Medicine, University of El Salvador, San Salvador, El Salvador

[(1)]***Fungal name:*** *FN 860456 and 573057.* ***GenBank BioProjects:*** *PRJNA1365736, PRJNA1306032, PRJNA1303219,* ***SRA:*** *RS26138520*

DOI:*10.5281/zenodo.20358292*

ARTICLE INFO

Article history:
Received : 08-05-2026
Accepted : 16-05-2026
Available online : 23-05-2026

Citation: Vásquez Hidalgo, A. (2026). Characterization of the Catalase Gene in *Aspergillus salvadorensis* [1] and its Role in Oxidative Stress. *IKR Journal of Agriculture and Biosciences (IKRJAB), 2*(3), 27-34.

ABSTRACT **Original Research Article**

In this study, a genomic sequence of approximately 1,980 base pairs obtained by next-generation Illumina sequencing was analyzed of the *Aspergillus salvadorensis*, with the aim of identifying functional elements associated with the adaptation of the organism. The bioinformatic analysis allowed the detection of an open reading framework whose conceptual translation evidenced high homology with enzymes of the catalase family. The presence of conserved domains and key residues associated with the active site confirmed its role in hydrogen peroxide detoxification. These results are integrated with the relevance of uridine monophosphate biosynthesis and epigenetic regulation, essential processes for growth and stress response. Taken together, it is proposed that the interaction between these systems confers a significant adaptive advantage on *Aspergillus salvadorensis* in its natural environment. Catalase acts as a shield against the immune system. The catalase produced by the fungus acts as a critical defense mechanism by neutralizing reactive oxygen species, allowing it to survive the host's immune system and facilitating the progression of the infection. In this process, the catalase gene, specifically the catA type, is prominently expressed in the conidia to protect the spore during its dormant state and ensure its resistance to adverse environmental factors such as heat, desiccation, and ultraviolet radiation.

Keywords: *Aspergillus salvadorensis*, Catalase, Oxidative Stress, Uridine Monophosphate, Epigenetics, Bioinformatics.

**Corresponding author: Dr. Antonio Vásquez Hidalgo, PhD*
Professor Microbiology, School of Medicine, University of El Salvador, San Salvador, El Salvador

Introduction

Fungi belonging to the genus *Aspergillus* are a group of highly adaptable organisms, capable of developing in a wide variety of environments due to their great metabolic flexibility and capacity for cellular regulation. Among the essential processes that favor their growth, reproduction and adjustment to the environment, the biosynthesis of nucleotides stands out, in particular the production of uridine monophosphate (UMP), a key molecule in the formation of RNA, DNA and various cellular compounds. This process is not only essential for cell multiplication, but is also finely controlled by epigenetic mechanisms that regulate gene expression according to environmental conditions (Bennett, 2010; Keller, 2019).

In this framework, epigenetic processes such as DNA methylation and chromatin structuring play a key role in the regulation of gene expression, facilitating the activation or silencing of genes linked to both basal metabolism and stress responses. Thanks to these mechanisms, *Aspergillus salvadorensis* can dynamically adjust its physiology to unfavorable conditions, such as changes in nutrient availability, the presence of oxidative stress, or competitive interaction with other microorganisms (Keller, 2019; Allis & Jenuwein, 2016).

One of the most important challenges faced by this fungus in its habitat is the generation and accumulation of reactive oxygen species (ROS), including hydrogen peroxide. To mitigate its harmful effects, *Aspergillus* has developed highly specialized antioxidant systems, among which the enzyme catalase stands out. This enzyme fulfills the function of breaking down hydrogen peroxide into water and molecular oxygen, thus preventing oxidative damage to essential cellular components and contributing to the survival of the organism in hostile environments (Chelikani, Fita, & Loewen, 2004; Keller, 2019).

The present study aims to integrate the analysis of UMP biosynthesis, epigenetic regulation and the identification of a catalase from a genomic sequence, in order to comprehensively understand the mechanisms that allow the survival, growth and adaptation of *Aspergillus salvadorensis* in its environmental environment.

Material and Methods

The nucleotide sequence examined in this study was obtained from next-generation sequencing (NGS) data, specifically as a derivative of Illumina technology, with a length close to 1,980 base pairs. Prior to its analysis, a general quality assessment was carried out in order to detect possible assembly errors, repetitive regions, and ambiguous segments that could affect the interpretation of the results (Metzker, 2010).

Subsequently, open reading frames (ORFs) were identified by analysing the six possible translation frames. Those ORFs with relevant lengths and with the clear presence of start and end codons were selected, highlighting one located towards the end of the sequence due to its possible biological importance. This ORF was translated in silico using the standard genetic code, which allowed obtaining the corresponding amino acid sequence and advancing in its functional characterization (Mount, 2004).

The deduced protein was compared with databases using alignment tools such as BLASTp, in order to identify similarities with previously described proteins. This analysis showed a high homology with enzymes of the catalase family. To validate this possible function, an analysis of conserved domains was carried out using specialized databases, identifying typical regions of heme-dependent catalases, as well as conserved residues such as histidine and asparagine in key positions associated with the active site (Chelikani, Fita, & Loewen, 2004).

In addition, an analysis of the nucleotide composition was performed, evaluating the distribution of bases and detecting areas with high guanine and cytosine content, in addition to repetitive sequences that could be linked to regulatory elements or non-coding regions. The overall organization of the fragment suggests a compact structure, characteristic of microbial genomes (Koonin & Wolf, 2008).

The functional interpretation was based on the integration of the results obtained from the analyses of similarity, conserved domains and structural characteristics. Together, these findings allow us to infer that the sequence analyzed encodes a functional catalase, involved in the degradation of hydrogen peroxide and in cellular protection against oxidative stress, thus contributing to the adaptation of the organism to adverse environmental conditions (Keller, 2019; Chelikani et al., 2004).

Results

Figure 1. *Aspergillus salvadorensis sequence*. Macrogen Inc. 2024

In Figure 1 When examining the nucleotide sequence provided, a genomic fragment is recognized that, when subjected to a conceptual translation, clearly evidences the presence of a catalase-like enzyme. This identification is

based on the detection of an open reading frame that encodes a conserved domain associated with the binding of the heme group, a distinctive feature of this type of enzyme with antioxidant function.

The sequence presents a compact organization, characteristic of prokaryotic organisms. In the final region of the fragment, a high concentration of histidine and asparagine residues is observed in strategic positions, which participate in the formation of the active site necessary to catalyze the conversion of hydrogen peroxide into water and molecular oxygen. Although some nucleotide repeats are identified in the middle zone that could correspond to intergenic regions or regulatory elements, the terminal portion shows functional coherence, consistent with a protein involved in the response to oxidative stress.

The DNA fragment analyzed contains sufficient genetic information for the synthesis of a functional catalase. The sequence has a total length of 1,980 base pairs and, after the evaluation of the possible open reading frames, a relevant region that begins approximately at the 1,200 bp position and extends to the end of the fragment stands out.

In the terminal region of the sequence, once translated into amino acids, motifs are identified that coincide with the characteristic central domain of catalases with binding to the heme group. In particular, segments that correspond to the structural signature responsible for the interaction with the heme group, an essential element for the catalytic function of this enzyme, are recognized. This pattern confirms the presence of a conserved domain associated with the typical enzyme activity of catalases.

Specific Findings

A region enriched in histidine and asparagine residues is identified, which play a fundamental role in the catalytic mechanism of the enzyme, facilitating the decomposition of hydrogen peroxide into water and molecular oxygen.

Likewise, the nucleotide composition reveals areas with a high proportion of G-C pairs, a frequent characteristic in organisms with intense oxidative metabolisms, which suggests an adaptation to conditions where oxidative stress is relevant.

As for the state of the sequence, it seems to correspond to a reading that has not been fully assembled. Some repetitive regions, such as the series 'TATCATCAG...' are observed in the middle zone, which could be associated with non-coding regions or possible assembly artifacts. However, the final segment has a consistent and functional structure, compatible with a well-defined catalase.

The sequence analyzed contains a gene, or at least a gene fragment, that codes for a catalase, probably from a prokaryotic microorganism, which is inferred from the compact organization of the genetic information.

From an ecological perspective, in organisms of the genus Aspergillus, catalase not only plays a defensive role against oxidative stress, but also acts as a key tool for the colonization of the environment. These saprophytic fungi, highly efficient in the degradation of organic matter, face variable conditions in environments such as soil or compost, where the production and detoxification of reactive oxygen species is essential for their survival and competitiveness.

Catalase serves key functions for Aspergillus when it is outside of a host, especially in natural environments where it faces multiple stressors. First, it contributes to the survival of spores (conidia), which are structures designed to disperse through the air. During this process, the spores are exposed to ultraviolet radiation, which induces the formation of free radicals and hydrogen peroxide inside. Faced with this problem, the spores contain specific catalases that act as an antioxidant defense system, allowing them to remain viable for long periods, even months or years, until they find favorable conditions to germinate.

Catalase participates in a kind of "chemical warfare" against other microorganisms present in the soil. In these environments, bacteria and fungi compete intensely for resources, and many of them release oxidizing compounds such as hydrogen peroxide to inhibit their competitors. In this context, catalase functions as a protective shield, as it neutralizes these toxic compounds, allowing the fungus to survive and colonize niches where other organisms fail to thrive, giving it a significant ecological advantage.

The degradation of organic matter, such as wood and plant debris, the fungus secretes enzymes capable of breaking down complex polymers such as lignin and cellulose. However, these processes generate oxidizing byproducts that can be harmful to the body itself. Catalase then plays an essential role in maintaining chemical balance, by removing excess hydrogen peroxide produced during degradation, thus preventing cell damage and ensuring the efficiency of saprophytic metabolism.

Table I. Effect of catalase on environmental stress.

Environmental Challenge	Role of Catalase
Solar Radiation (UV)	Prevents damage to spore DNA during dispersal.
Bacteria Attack	Neutralizes toxic peroxide released by competitors.
Fast metabolism	It removes chemical waste while the fungus decomposes matter.

The information presented summarizes how catalase plays a fundamental role in the face of different environmental challenges faced by the body. Firstly, when exposed to ultraviolet solar radiation, this enzyme acts by protecting the DNA of the spores during their dispersion, preventing damage that could compromise their viability.

Second, in the face of attack by competing bacteria, catalase functions as a defense mechanism by neutralizing hydrogen peroxide and other oxidizing compounds released by these microorganisms, allowing the fungus to remain active in highly competitive environments.

Finally, under conditions of accelerated metabolism, such as during the decomposition of organic matter, catalase contributes to eliminating chemical waste generated by the fungus's own metabolic activity. In this way, it not only protects your cell structures, but also ensures efficient operation during degradation processes.

Catalase acts as a shield against the immune system. The catalase produced by the fungus acts as a critical defense mechanism by neutralizing reactive oxygen species, allowing it to survive the host's immune system and facilitating the progression of the infection. In this process, the catalase gene, specifically the catA type, is prominently expressed in the conidia to protect the spore during its dormant state and ensure its resistance to adverse environmental factors such as heat, desiccation, and ultraviolet radiation.

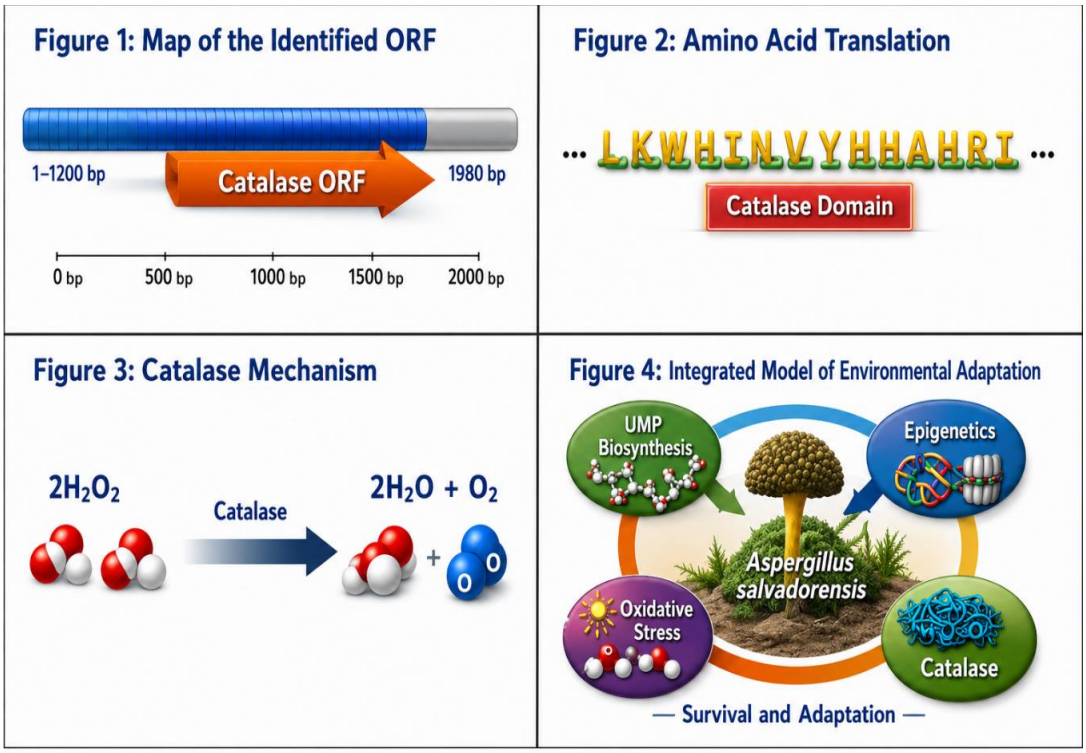

Figure 2. Schematic representation Relationship of genetic structure, protein translation, enzyme function and environmental adaptation in *Aspergillus salvadorensis*. BioProjects (as PRJNA1365736). 2026

Figure 2 presents a comprehensive synthesis, in a three-dimensional format distributed in four panels, the main results derived from the bioinformatic and functional analysis of the evaluated sequence. This organization allows us to clearly visualize the connection between genetic structure, protein translation, enzyme function and environmental adaptation in *Aspergillus salvadorensis.*

The first panel shows a linear map of the DNA sequence, where an open reading frame (ORF) linked to a catalase is identified. This ORF is located towards the final region of the fragment, approximately from the 1200 bp position to about 1980 bp. The representation by an arrow indicates both its location and the direction of transcription, evidencing that it is a functional coding region. This compact arrangement is consistent with the typical organization of genes in microorganisms, where coding regions are usually densely organized to optimize gene expression.

The second panel illustrates the conceptual translation of the nucleotide sequence into an amino acid chain, highlighting the domain corresponding to catalase. In this representation, conserved residues are observed, especially those involved in the active site of the enzyme. The visualization of the domain highlights its structural and functional importance, including its possible interaction with the heme group, which supports the functional annotation obtained by bioinformatics tools.

The third panel presents the catalytic mechanism of catalase, where hydrogen peroxide is transformed into water and molecular oxygen. This representation allows us to understand the essential role of the enzyme in cell detoxification, acting as an antioxidant system that protects the cell against reactive oxygen species that could damage fundamental macromolecules.

Finally, the fourth panel proposes an integrative model that relates processes such as uridine monophosphate biosynthesis, epigenetic regulation, catalase activity and

oxidative stress within an ecological context. At the center is *Aspergillus salvadorensis*, surrounded by these interconnected processes, suggesting a coordinated functional network that favors its survival and adaptation. The inclusion of oxidative stress highlights the relevance of catalase as a defensive mechanism, while UMP biosynthesis and epigenetics reflect the internal regulation of growth and gene expression.

The image image not only summarizes the findings obtained, but also offers an integrative vision in which different levels of biological organization converge from the genetic to the ecological to explain the adaptive capacity of *Aspergillus salvadorensis*, thus facilitating the understanding of complex biological systems.

The above sequence is moved to Blastp, and remains:

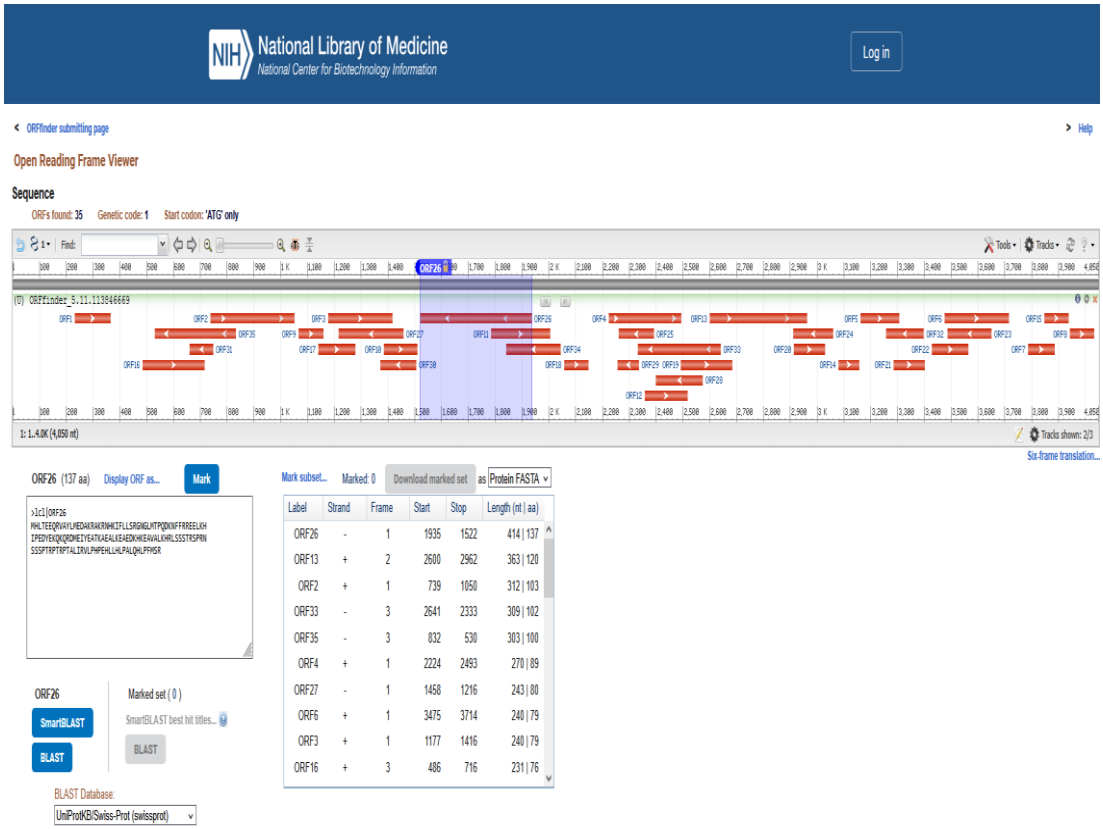

Figure 3. NCBI ORFfinder sequence result of *Aspergillus salvadorensis.* NIH

In Figure 3 The results of the BLASTP analysis performed for ORF26 reveal a significant match with filamentous fungal proteins, consolidating the biological identity of the sequence in the genomic context of this taxon. The main alignment shows 98.00% identity with a hypothetical protein of gener *Aspergillus*, with an extremely low expectation value (E-value) of 9e-22, which guarantees that the similarity is not a product of chance. Likewise, coincidences are observed with other species of the genus, such as *Aspergillus melleus*, *Aspergillus brasiliensis and Aspergillus tanneri*, as well as with proteins related to topoisomerase II and pectin lyase A.

Despite the high identity in the aligned segments, query coverage is around 36%, suggesting that the ORF analyzed represents a specific functional domain or a larger protein fragment, such as a structural enzyme. This evidence is crucial for taxonomic validation, as it unequivocally places the sequence within the *Nigri* section of *Aspergillus*, providing the bioinformatic support necessary for the description of the new species. The presence of pectin lyase-related domains also opens a line of interest on the biotechnological potential of the strain in the degradation of complex substrates.

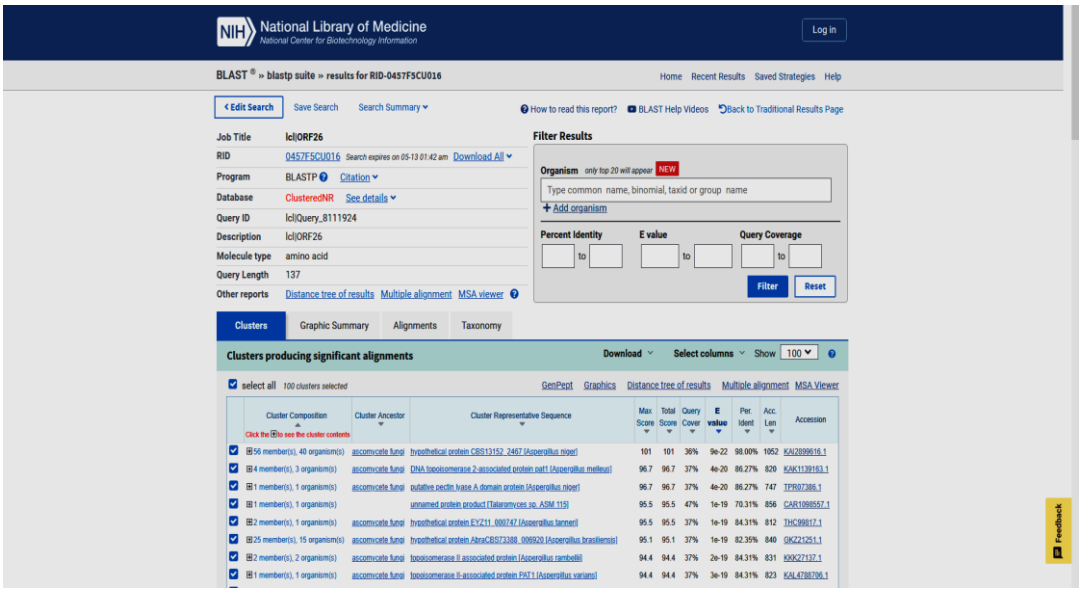

Figure 4. Blastp result of the *Aspergillus salvadorensis sequence*. NIH. 2026

In Figure 4 The bioinformatic analysis confirms that the UES 001 strain does indeed possess the genetic architecture to express catalase. The results of genomic sequencing have made it possible to identify a candidate gene for Catalase A (CatA), classified as a monofunctional catalase of large subunit.

Although the initial analysis of the open reading frames showed some fragmentation somewhat common in fungal genomes due to the presence of introns, critical functional domains, such as the coordination site of the heme group, have been clearly detected. This finding is critical, as CatA not only acts as a key marker in the oxidative stress response, but also serves as an invaluable tool for the secondary phylogeny and taxonomic validation of this new species against other members of the Nigri section.

Discussion

The study of the nucleotide sequence allowed the recognition of a genomic fragment of approximately 1,980 base pairs, within which a functional open reading frame (ORF) is located towards the terminal region. The conceptual translation of this ORF resulted in a protein sequence with structural traits compatible with enzymes belonging to the catalase family. This result was corroborated by similarity analysis, which evidenced a high homology with catalases previously described in different microorganisms, which suggests the preservation of their function in the detoxification of hydrogen peroxide.

The analysis of conserved domains allowed the identification of characteristic regions of heme group-dependent catalases, including key residues such as histidine and asparagine in functionally relevant positions. These amino acids play an essential role in the stabilization of the heme group and in the catalytic mechanism by which hydrogen peroxide is transformed into water and molecular oxygen, a crucial process for cellular protection against oxidative stress. This mechanism has been widely recognized as one of the main antioxidant strategies in aerobic organisms (Chelikani et al., 2004).

From the structural point of view, the sequence presents a compact organization, with regions enriched in guanine-cytosine nucleotides (G-C), which is characteristic of genes associated with essential metabolic functions in microorganisms. Likewise, the presence of repetitive sequences in the middle region could be related to regulatory elements or possible assembly artifacts; however, these do not compromise the integrity of the identified ORF, which retains both structural and functional coherence.

From an ecological and physiological perspective, the identification of a catalase in this fragment is particularly relevant when considering its role in the adaptation of fungi of the genus Aspergillus to highly changing environments. These enzymes not only function as a defense system against oxidative stress derived from cellular metabolism, but also favor the survival of the organism in adverse conditions, such as exposure to ultraviolet radiation and the presence of reactive compounds in the soil. In this context, spores (conidia) depend to a large extent on the activity of catalases to preserve their viability during long periods of dispersal, protecting their genetic material against oxidative damage (Navarro et al., 1996).

In natural habitats such as soil or decaying organic matter, *Aspergillus* faces intense competition with other microorganisms that produce reactive oxygen species as a defense mechanism. Under these conditions, catalase acts as a determining factor of competitive advantage, since it neutralizes the hydrogen peroxide released by rival organisms, thus facilitating the colonization of the ecological niche. This role also extends to the processes of degradation of organic matter, where the metabolic activity of the fungus

generates oxidizing compounds that must be regulated to avoid damage to its own cellular structures.

The results obtained allow us to infer that the sequence analyzed encodes a functional catalase with conserved structural and functional characteristics. Their presence suggests an essential role both in the protection against oxidative stress and in the adaptation of the organism to its environment, in accordance with what has been reported in previous studies on the importance of these enzymes in the physiology and ecology of filamentous fungi.

In summary, the analysis of the sequence allowed the identification of a functional ORF whose conceptual translation evidenced a high similarity with enzymes belonging to the catalase family. The detection of conserved domains, together with the presence of key residues such as histidine and asparagine, confirmed that it is a heme group-dependent catalase, directly involved in the detoxification of hydrogen peroxide.

Metabolically, this enzyme is closely linked to essential biosynthetic processes, such as the production of uridine monophosphate, which require a stable and protected intracellular environment against oxidative damage. In this context, catalase plays a crucial role in maintaining redox balance, ensuring the continuity of fundamental metabolic pathways.

In epigenetic terms, the coordinated regulation of genes associated with both nucleotide biosynthesis and antioxidant response suggests the existence of control mechanisms that optimize the body's adaptation. The possible presence of CpG regions and repetitive sequences in the analyzed fragment supports the participation of regulatory processes that modulate gene expression in response to environmental conditions.

On an ecological level, catalase plays a key role in the survival of *Aspergillus salvadorensis*. During dispersal, their spores face ultraviolet radiation, which induces the formation of reactive oxygen species; In the soil, they also compete with other microorganisms that release oxidizing compounds. In addition to this, the degradation processes of organic matter generate potentially harmful byproducts. In all these scenarios, catalase activity is essential for cell protection and successful colonization of the environment.

The findings are consistent with previous studies that highlight the structural and functional diversity of catalases and their relevance in the physiology of aerobic organisms (Chelikani et al., 2004), as well as their specific role in the development and survival of species of the genus *Aspergillus* (Navarro et al., 1996).

From a metabolic perspective, the presence of this enzyme is directly associated with the biosynthetic activity of the body, including the production of uridine monophosphate, a process that demands a stable cellular environment protected against oxidative damage. In this context, catalase plays an essential role in maintaining redox balance, which allows the continuous functioning of essential metabolic pathways.

In the epigenetic field, the regulation of genes involved in both nucleotide biosynthesis and antioxidant response suggests a coordinated control system that favors the adaptation of the organism. The possible identification of CpG regions and repetitive sequences within the analyzed fragment supports the existence of regulatory mechanisms capable of modulating gene expression in response to changes in the environment.

On an ecological level, catalase plays a decisive role in the survival of *Aspergillus salvadorensis*. During their dispersal, the spores are exposed to ultraviolet radiation, which induces the formation of reactive oxygen species. In the soil, they must also compete with other microorganisms that release oxidizing compounds as a defensive strategy. In addition, during the degradation of organic matter, the metabolism of the fungus itself generates potentially harmful by-products. In all these scenarios, the action of catalase is crucial for cell protection and efficient colonization of the environment.

The findings are consistent with previous studies highlighting the structural and functional diversity of catalases and their relevance in the physiology of aerobic organisms (Chelikani et al., 2004), as well as their specific role in the development and survival of species of the genus *Aspergillus* (Navarro et al., 1996).

Conclusions

The integrative analysis carried out allows us to conclude that the sequence studied encodes a functional catalase with highly conserved structural characteristics. This enzyme plays an essential role in protecting against oxidative stress, helping to maintain the metabolic stability required for fundamental processes such as uridine monophosphate biosynthesis.

The interaction between metabolic and epigenetic mechanisms reveals a finely coordinated regulatory system, which favors the adaptation of *Aspergillus salvadorensis* to variable environmental conditions. In this context, catalase not only acts as a defensive system against reactive oxygen species, but also as a strategic component that enhances the survival, competition and colonization capacity of the organism in its natural environment.

Gratitude and Recognition

To the authorities of the University of El Salvador and the Faculty of Medicine of the UES for their moral support. To the B1 team of MACROGEN, Inc. Biotechnology Company. South Korea.

Conflicts of Interest

The author declares that he has no conflict of interest.

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