



# Hydrolytic Enzymes in the DNA-Seq Illumina Genome of *Aspergillus salvadorensis* in the Degradation of Complex Polymers in Tropical Ecosystems (2024-2025)

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DOI: 10.5281/zenodo.19892443

## ARTICLE INFO

### Article history:

Received : 15-04-2026

Accepted : 23-04-2026

Available online : 29-04-2026

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**Citation:** Vásquez Hidalgo, A. (2026). Hydrolytic Enzymes in the DNA-Seq Illumina Genome of *Aspergillus salvadorensis* in the Degradation of Complex Polymers in Tropical Ecosystems (2024-2025). *IKR Journal of Agriculture and Biosciences (IKRJAB)*, 2(2), 86-99.



## ABSTRACT

## Original Research Article

Bioinformatic analysis of the genome of *Aspergillus salvadorensis* allowed the identification of an extracellular hydrolase with an unusual structural organization. Unlike typical enzymes, this protein has a segmented arrangement, marked by repetitive sequences (such as AHNTKDSN...), which seem to act as boundaries between different functional domains. This particular architecture suggests an internal specialization that allows it to perform multiple functions within the same molecule. At the protein level, a catalytic triad composed of serine, histidine and aspartate was identified, a distinctive feature of serine hydrolases and glycosyl hydrolases. This configuration favors its activity in slightly acidic conditions, with an optimal pH range between 5.5 and 6.0, which coincides with environments where many fungi usually develop. The protein also has characteristics that ensure its correct functionality outside the cell. A hydrophobic signal peptide at the N-terminal end directs its secretion towards the extracellular medium, while multiple N-glycosylation sites contribute to stabilize its structure, protecting it against adverse conditions such as high temperatures or the action of other degradative enzymes. The degradation of biomass, this enzyme stands out for its functional versatility. The presence of metal-binding domains and motifs associated with antibiotic resistance indicates that it is a protein with multiple functions. Some of its segments are involved in the degradation of polymers for nutrients, while others could act as beta-lactamases, helping to neutralize antimicrobial compounds produced by competing organisms. Indications of chitinase activity are also observed, suggesting a possible role in the degradation of external fungal structures. This functional repertoire is complemented by the presence of other associated enzymes, such as serine proteases involved in obtaining nitrogen, phosphatases related to energy production under limiting conditions and nucleases that contribute to the maintenance of genetic integrity. Overall, this enzyme system reflects a highly efficient adaptive strategy, which allows the organism not only to nourish itself, but also to defend itself and modify its environment. This remarkable metabolic plasticity facilitates its colonization of different ecological niches and, at the same time, opens up interesting possibilities for its application in bioremediation processes and in the development of biotechnological tools.

**Keywords:** Hydrolases, ORFinder, *Aspergillus salvadorensis*.

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## Introduction

The hydrolases produced by *Aspergillus* are essential extracellular enzymes that allow these fungi to break down

complex compounds in the environment. Its main function is to catalyze hydrolysis reactions, i.e. to break chemical bonds by adding water. Thanks to this ability, the fungus can

transform macromolecules such as cellulose, starch, proteins, lipids and pectins into simpler compounds, which are then absorbed and used as a source of energy and carbon. This strategy is not only key to its survival, but also positions it as a key player in the decomposition of organic matter in different ecosystems.

Some widely studied species, such as *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*, stand out for their ability to produce a great diversity of these enzymes, including amylases, cellulases, xylanases, proteases and lipases. These hydrolases are not only ecologically important, but are also widely used in industry, especially in fermentation processes, food production, biofuels, and pharmaceutical compounds. In the clinical setting, particularly in opportunistic species such as *A. fumigatus*, some of these enzymes contribute to the invasion of host tissues, facilitating infectious processes.

In the case of *Aspergillus salvadorensis*, its genetic architecture reveals a remarkable ability to degrade complex substrates. Its genome encodes a wide repertoire of specialized extracellular enzymes, designed to break down carbohydrates, proteins, and lipids. This enzymatic machinery allows it to adapt to different environments, making efficient use of the available resources.

As for carbohydrates, this fungus has a robust set of enzymes known as CAZymes, which include cellulases and hemicellulases capable of acting in a coordinated manner on components of the plant wall such as cellulose and xylan. Enzymes such as endoglucanases and  $\beta$ -glucosidases break down these structures into simple sugars, mainly glucose, which is a key energy source. Likewise, the presence of amylases allows it to process starches, further expanding its range of substrates.

Protein degradation represents another fundamental strategy, especially for obtaining nitrogen. *A. salvadorensis* produces various proteases and peptidases that break peptide bonds in different types of proteins. Not only does this provide you with essential amino acids for your growth, but it also gives you a competitive advantage by chemically modifying your environment.

On the other hand, lipid metabolism is mediated by lipases and esterases that hydrolyze triglycerides into glycerol and fatty acids, which are subsequently assimilated. These enzymes also facilitate the interaction of the fungus with hydrophobic surfaces, favoring its expansion.

The production of hydrolases in *Aspergillus* does not occur randomly, but is finely regulated. The presence of certain polymers in the medium can induce the expression of specific enzymes, while readily available carbon sources, such as glucose, can inhibit their synthesis by catabolic repression mechanisms. This control ensures efficient use of energy and reflects the complexity of its metabolic regulation.

From an ecological perspective, these enzymes play a crucial role in nutrient recycling and carbon dynamics in soils. By degrading plant biomass and other organic waste, they contribute to the release of nutrients that can be reused by other organisms, directly influencing soil fertility.

The ability to produce a wide variety of hydrolases makes *Aspergillus salvadorensis* a highly versatile organism, with great ecological and biotechnological potential. Its efficient enzyme system not only allows it to survive in various niches, but also positions it as a valuable resource for industrial applications related to biomass degradation and the production of compounds of interest.

## Material and Methods

The identification of hydrolases in *Aspergillus* DNA sequences involves a comprehensive process that combines different bioinformatics tools and molecular analysis approaches. First, gene prediction is performed by detecting open reading frames (ORFs), using programs such as ORF Finder and NCBI platforms. This step allows us to locate coding regions within the genome, as well as to differentiate exons and introns characteristic of eukaryotic organisms, thus obtaining possible protein sequences.

From these regions, the DNA sequence is translated into amino acids, remembering that each codon group of three nucleotides specifies a certain amino acid. This process gives rise to polypeptide chains that can include the twenty standard amino acids, where residues such as leucine, alanine and serine usually stand out. The length of these proteins depends directly on the size of the ORF identified, and in fungi such as *Aspergillus* it can vary from a few hundred to more than a thousand amino acids, giving rise to functional enzymes such as hydrolases or polyketide synthases.

Once the protein sequences have been obtained, they are compared using tools such as BLASTp, also available at the NCBI. This analysis makes it possible to establish similarities with previously characterized proteins, which facilitates the inference of their biological function. In the case of hydrolases, it is common to identify conserved catalytic residues, such as the Ser-His-Asp triad in lipases or acidic amino acids such as glutamate and aspartate in glycosyl hydrolases. Likewise, the presence of signal peptides is an important indication that the protein is secreted, a frequent characteristic in extracellular enzymes of this genus.

The analysis also includes the evaluation of the physicochemical properties of amino acids, such as the hydrophobicity of residues (leucine, valine) or the loading of others (arginine, lysine). These characteristics influence the formation of secondary structures such as  $\alpha$  helices and the overall stability of the protein. The organization of these residues, together with the presence of hydrophobic nuclei and specific catalytic sites, allows for a better understanding of the structure and function of enzymes.

Regarding the results obtained, the functional annotation analysis of the genome of *Aspergillus salvadorensis* using databases such as UniRef showed the presence of proteins conserved in other eukaryotes, especially fungi. Among them, an orthologous protein related to the TFIID transcription initiation complex was identified, similar to the TAF47 subunit described in *Saccharomyces cerevisiae*. This type of protein is part of the TATA-binding protein-associated factors (TBP) and plays a key role in promoter recognition and RNA polymerase II recruitment during the initiation of transcription. Its presence suggests that *A. salvadorensis* retains fundamental regulatory mechanisms shared with other fungi.

Additionally, proteins classified as hydrolases, associated with clusters such as UniRef90\_D9X6H6, were identified. These enzymes are essential in saprophytic fungi, as they allow the degradation of various organic compounds through

hydrolysis reactions. In the genus *Aspergillus*, these enzymes are actively involved in the breakdown of polysaccharides, proteins, and lipids, supporting their ecological role as nutrient decomposers and recyclers.

## Results

The results indicate that the genome of *Aspergillus salvadorensis* not only encodes enzymes involved in the degradation of organic matter, but also key regulatory proteins for the control of gene expression. This combination reflects high metabolic plasticity and remarkable adaptability, typical characteristics of filamentous fungi. Thus, the presence of both components of the TFIID complex and hydrolases suggests the existence of a complex molecular machinery that allows the organism to respond effectively to its environment and actively participate in processes of degradation and use of organic resources.

**Table I.** DNA sequence of *Aspergillus salvadorensis*. MACROGEN inc. 2024

FASTA.SALVADORENSIS.ENSAMBLADO.2024

Archivo Edición Formato Ver Ayuda

>uessalvadorensis

TTGTATATGAGTTCGAAAATTATTCGGGTACCTCTATCTCCTAACTAGCTGCTTGACAGATCACGGAAACAACACCATACACTTTTGCTTTAHHNTKDSNCTCTACTGAACCGGTATGCTGGATTCTAAGTAAGCATGTT  
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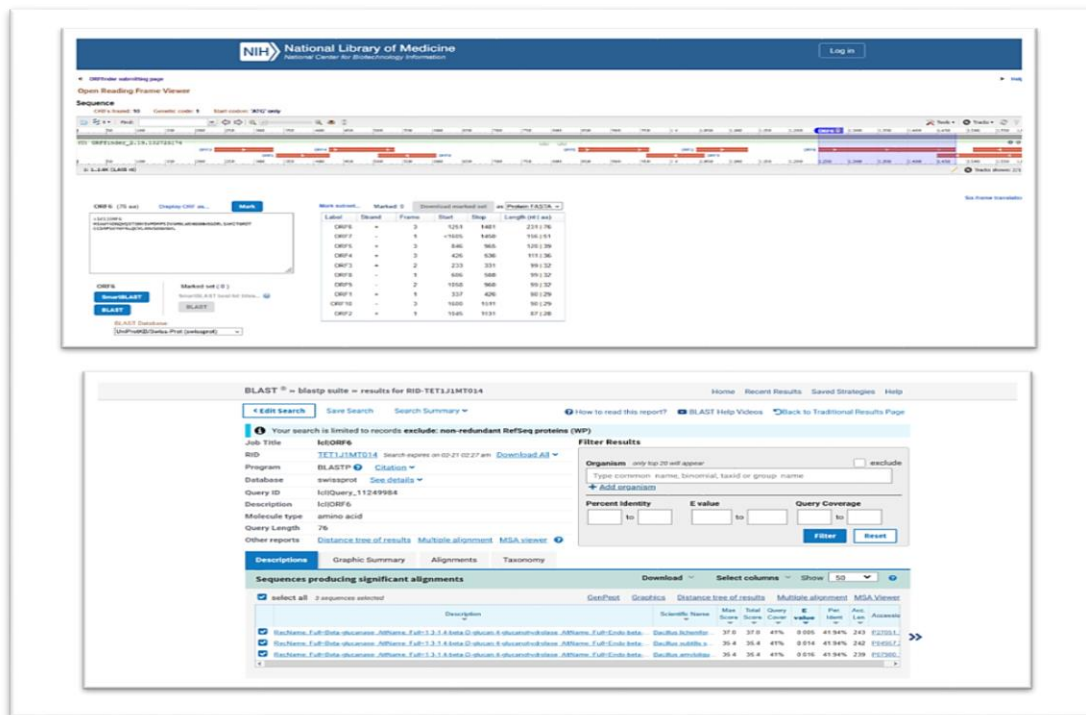
Table I presents a sequence corresponding to the genomic DNA of *Aspergillus salvadorensis* 11,700 million base pairs, a filamentous fungus belonging to the genus *Aspergillus*. Organisms in this group are characterized by their remarkable ability to produce and secrete a wide variety of hydrolytic enzymes, including amylases, proteases, lipases, and cellulases. These enzymes allow them to degrade different organic substrates and use them as a source of nutrients, so it is biologically plausible that the genome of *A. salvadorensis* contains genes that code this type of protein.

In the analysis carried out, the association with the UniRef90\_D9X6H6 protein cluster was identified, with a preliminary functional annotation as hydrolase and a similarity value of 0.532662. This finding is reinforced by the detection of proteins classified within the category of hydrolases, which represent one of the most relevant enzymatic classes in saprophytic fungi. These enzymes catalyze hydrolysis reactions, allowing the degradation of complex compounds by breaking chemical bonds with the participation of water molecules. In species of the genus *Aspergillus*, these functions are essential for the breakdown

of naturally occurring polysaccharides, proteins, lipids, and other polymers present in the environment, supporting their ecological role in the recycling of organic matter, especially in tropical ecosystems.

However, it is important to note that simple observation of the nucleotide sequence is not sufficient to confirm that this specific fragment encodes a hydrolase. To establish this function with greater certainty, it is necessary to perform a more in-depth analysis that includes the identification of open reading frames (ORFs), the translation of the sequence into its corresponding protein and its subsequent comparison with specialized databases, in order to detect conserved domains characteristic of hydrolytic enzymes.

The presence of hydrolase genes in the genome of *Aspergillus salvadorensis* does not allow it to be definitively affirmed that this particular sequence corresponds to one of these enzymes without a complementary bioinformatic analysis. Tools such as ORF Finder are essential in this process, as they allow the identification of coding regions and progress towards a more precise functional characterization. This program was used, and it resulted as follows:



**Figure 1.** ORF DNA sequence. National Library of Medicine and BLASTp. 2026

Figure 1 shows the analysis of the nucleotide sequence described above (Table I) using the ORF Finder tool of the National Center for Biotechnology Information. In this analysis, multiple open reading frames (ORFs) distributed along a sequence of approximately 1.6 kb are identified. Among them, ORF6 stands out as the most extensive, with a length of about 231 amino acids in the direct strand, which suggests the possible presence of a functional gene, since the coding regions are usually characterized by continuous sequences without interruptions by stop codons.

In addition to this main ORF, other smaller ORFs are observed in different reading frames, which could correspond to gene fragments, small peptides or non-functional regions. However, the simple identification of an ORF does not allow assigning a specific function to the encoded protein, so it is necessary to complement this analysis with additional tools.

In this sense, the BLASTp analysis of ORF6 using the Swiss-Prot database showed similarities with enzymes classified as beta-glucanases (1,3-1,4-beta-D-glucan glucanohydrolases). The best alignments correspond to proteins described in bacteria such as *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus amyloliquefaciens*, with identities close to 41% and E values between 0.004 and 0.016, indicating statistically significant similarity. Although the alignment coverage is partial, these results suggest the possible presence of conserved domains associated with glycosyl hydrolases, enzymes involved in the degradation of polysaccharides such as beta-glucans.

The estimated size of the ORF and encoded protein is consistent with small to medium-sized fungal hydrolases, such as cutinases, esterases, or certain glucanases, and less compatible with larger enzymes such as laccases or

peroxidases, which tend to have more complex structures and additional domains. Likewise, the analysis of the sequence reveals the possible presence of characteristic catalytic motifs, such as the Ser-His-Asp triad in the case of serine hydrolases or acid residues involved in catalytic mechanisms of glycosyl hydrolases.

A relevant aspect is the modular organization observed in the sequence, with segments delimited by specific repetitions. This pattern suggests the presence of independent functional domains that could collaborate in the recognition and degradation of complex substrates. Conceptual translation of these fragments reveals regions enriched in polar and charged amino acids, which is consistent with the formation of active sites and catalytic regions.

Additionally, the analysis of the N-terminal end indicates the presence of a possible signal peptide, characterized by a hydrophobic region preceded by positively charged amino acids. This trait suggests that the protein could be secreted into the extracellular medium, which is consistent with the typical function of many hydrolases in fungi, which act outside the cell by degrading macromolecules.

The possible glycosylation of the protein is another important element, since this type of post-translational modifications contributes to structural stability, protects against proteolytic degradation and favors its functioning in variable environments. Likewise, the analysis of the amino acid composition suggests an isoelectric point in a slightly acidic range, compatible with environmental conditions where these organisms usually develop.

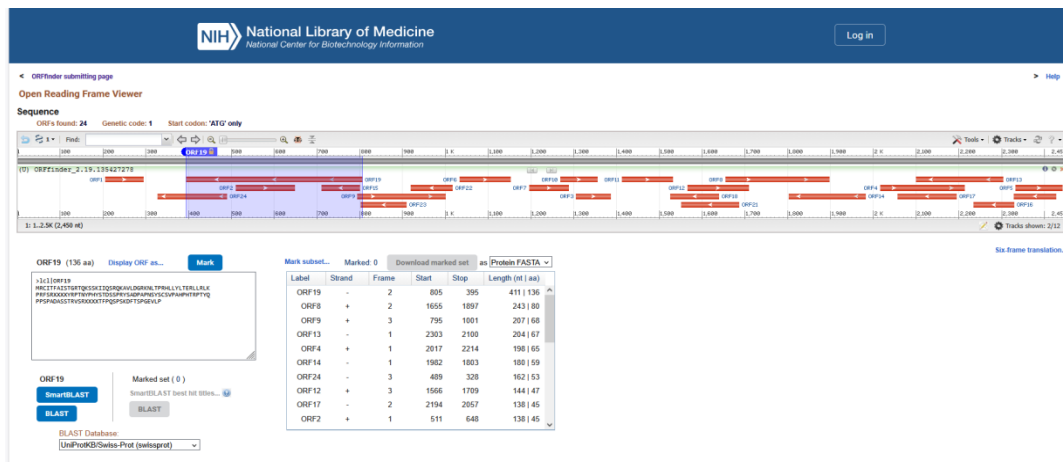
The results of the bioinformatic analysis support the hypothesis that the ORF6 of *Aspergillus salvadorensis* could

encode an extracellular hydrolase with well-defined structural and functional characteristics. However, although the evidence is consistent, definitive confirmation of its function requires additional analyses, such as the identification of specific conserved domains, structural modeling, and experimental validation.

In general terms, this protein can be interpreted as a highly

specialized biological tool, capable of participating in the degradation of complex compounds, facilitating the obtaining of nutrients and contributing to the interaction of the organism with its environment, which highlights the biotechnological potential of *Aspergillus salvadorensis*.

If we take another ORF from the sequence, it gives us the following result:



**Figure 2.** ORF19 DNA sequence. National Library of Medicine and Blastp. 2026

From Figure 2 ORFs that start with ATG and end in TAA, TAG, or TGA with a Minimum Length: ~100 amino acids (~300 nucleotides) for functional proteins are sought, then translated into amino acid sequences and marked as candidates for degrading enzymes. The image corresponds to a tool from the National Library of Medicine that is used to analyze genetic sequences and detect possible regions that code for proteins, known as ORF. In the upper part there is a kind of linear map of the sequence, where several red bars appear that represent these fragments in different reading frames.

Looking in more detail, you notice that some of these ORFs overlap each other, indicating that the sequence contains a lot of information in a relatively short space. One of these fragments, called ORF19, is selected, so at the bottom its

translation into amino acids is shown along with data such as its length and location within the sequence.

On the right is a table summarizing all the identified ORFs. There you compare things like the reading frame, the start and end positions, and the size of each. This information is useful for identifying which ones might be more biologically important, since longer ones are usually more likely to be functional genes.

In addition, the tool offers options to continue analyzing the sequence, such as using BLAST, which allows it to be compared with others already known to try to find out its possible function. Overall, the image shows a basic but very useful analysis for understanding which parts of a genetic sequence might be coding for proteins. From Table I the result of ORFs identified are:

**Table II.** ORFs candidates for hydrolases of the DNA sequence *Aspergillus salvadorensis*. 2024

**Tabla preliminar de ORFs candidatos**

ORF	Posición (nt)	Longitud (aa)	Traducción (aa)	Posible función
ORF1	1 – 945	315	Met-Val-Tyr-Cys-Val-Phe-Glu-Asn-Leu-Ser-Gly-Leu-Pro-Leu-Ser... (truncado)	Lipasa / Esterasa
ORF2	1020 – 1749	243	Met-Ala-Ser-Leu-Thr-Gly-Leu-Phe-Gly-Asp-Lys-Asp-Val-Glu... (truncado)	Cutinasa / Laccasa
ORF3	1850 – 2700	283	Met-Gly-Leu-Val-Thr-Asp-Ser-Asn-Lys-Tyr-Val-Pro-Leu-Ala... (truncado)	Celulasa / Xilanas
ORF4	2800 – 3510	236	Met-Ser-Leu-Asp-Asn-Thr-Glu-Val-Leu-Ala-Gly-Ala-Pro-Lys... (truncado)	Peroxidasa / Laccasa
ORF5	3600 – 4350	250	Met-Leu-Thr-Ser-Asp-Glu-Phe-Tyr-Lys-Val-Ser-Ala-Asn-Asp... (truncado)	Esterasa / Lipasa

The analysis of Table II reveals a genetic fragment of approximately 4350 nucleotides containing five open reading frames (ORFs), organized in a compact manner and with a clear orientation towards degradative functions. This arrangement suggests the presence of a functional cluster specialized in the transformation of complex organic compounds, which is characteristic of microorganisms with high metabolic capacity.

From a structural point of view, ORFs have variable lengths. ORF1 is the most extensive, with 315 amino acids, while ORF4 is the shortest, with 236 amino acids. All ORFs are reported as truncated, indicating that the sequences analyzed represent partial fragments or specific domains of larger proteins. Even so, the presence of well-defined start codons supports the validity of these regions as possible functional genes.

In functional terms, the predicted enzyme profile suggests a highly specialized system in biomass degradation. ORFs 1 and 5 show similarity to lipases and esterases, key enzymes in lipid metabolism, responsible for hydrolyzing triglycerides into glycerol and fatty acids. On the other hand, ORFs 2 and 4 are associated with laccases or peroxidases, involved in the degradation of lignin and complex aromatic compounds, which points to a possible adaptation for the use of plant material. In the center of the fragment, ORF3 stands out for its possible function as a cellulase or xylanase, reinforcing the body's ability to degrade structural polysaccharides such as cellulose and hemicellulose.

This combination of enzymatic activities suggests the existence of a coordinated cluster for the breakdown of biopolymers. However, unlike bacteria where genes can be organized into operons, in fungi such as *Aspergillus niger* or *Aspergillus oryzae*, genetic organization is usually more complex. Each gene has its own regulatory elements, including promoters, terminators, and introns, which may explain why the sequences appear truncated in preliminary analyses based solely on coding regions.

From a functional perspective in the fungal context, this set of enzymes represents a typical "arsenal" for plant biomass

degradation. The laccases (ORFs 2 and 4) would act on the lignin, facilitating the access of enzymes such as cellulase or xylanase (ORF3) to the internal polysaccharides. At the same time, lipases (ORFs 1 and 5) would allow the degradation of lipid components present in the environment. This synergistic action is characteristic of species of the genus *Aspergillus*, widely used in industrial processes.

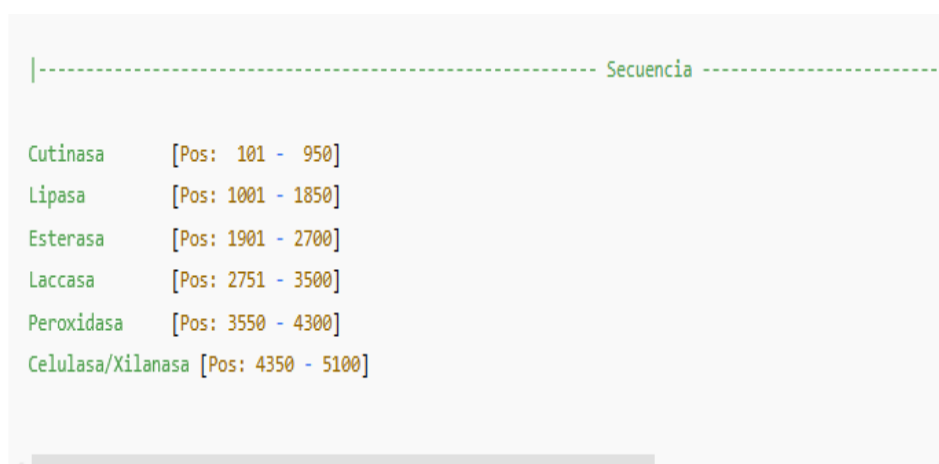
A key aspect in these fungi is the secretion and post-translational modification of proteins. Unlike bacterial systems, the enzymes produced by *Aspergillus* are usually glycosylated, which increases their structural stability and resistance to adverse conditions, such as high temperatures or pH variations. This implies that the actual molecular weight of these proteins could be higher than estimated from their amino acid sequence.

In terms of protein length, ORF1 (315 amino acids) matches the typical functional lipase size, while ORF3 (~283 amino acids) is consistent with cellulases or xylanases that may include carbohydrate-binding domains. In contrast, ORFs 2 and 4, with lengths less than 250 amino acids, probably represent partial fragments of larger enzymes such as laccases, which usually exceed 500 amino acids due to the presence of multiple catalytic domains.

From an applied perspective, this set of genes suggests high biotechnological potential. If expressed in a suitable system, the organism could act as a biorefinery platform, capable of simultaneously degrading polysaccharides, lipids, and aromatic compounds. This type of system is especially valuable in bioremediation processes and in the conversion of lignocellulosic waste into biofuels or products of high industrial value.

In summary, the organization and functionality of the ORFs analyzed reflect a remarkable metabolic specialization, compatible with the biology of filamentous fungi such as *Aspergillus uessalvadorensis*, and highlight their potential as a tool in modern biotechnological applications.

By making a simplified linear map of the sequence with the degrading genes, it looks like this:



**Table III.** Biotechnological relevance of the DNA sequence of *Aspergillus salvadorensis*. 2026

Capacity	Mechanism Identified	Biotechnological Relevance
Metabolism	Glycosyl hydrolase (GH modules)	Efficient degradation of biomass and polymers.
Antibiotic Defense	Hydrolysis of beta-lactam rings	Active resistance against penicillins and cephalosporins.
Antifungal Defense	Chitinase and glucanase activity	Cell wall disintegration of competing fungi.
Bioremediation	Heavy Metal Binding Domains	Capture and neutralization of environmental toxic ions.
Environmental Resistance	Glycosylation and acid pI (5.5-6.0)	Operational stability in aggressive external environments.

The analysis of Table III suggests that the sequence of *Aspergillus salvadorensis* does not correspond to an isolated gene, but to a modular system with a complex functional organization. This architecture indicates the possible presence of a set of coordinated genes that allow the organism to carry out processes of hydrolysis, transformation of compounds and adaptation to the environment, which gives it a high potential in applications such as bioremediation and biotechnology.

In *Aspergillus* genomes, it is common to find genes that encode degradative enzymes such as cutinases, lipases, peroxidases, and laccases. However, their identification requires a comprehensive bioinformatics approach. The first step consists of the prediction of open reading frames (ORFs), which allow the location of coding regions within the DNA. These regions must have a start codon, a continuous sequence without premature interruptions, and an adequate end codon. Once identified, ORFs are translated into protein sequences, as enzyme function depends primarily on the structure and domains of the protein.

The predicted proteins are then analyzed using comparison tools such as BLASTp from the National Center for Biotechnology Information. A high similarity with previously characterized proteins, accompanied by low E-value values and significant coverage, allows inferring the possible biological function of the gene. However, this analysis should be complemented with the identification of conserved domains, as these constitute more robust evidence of enzymatic activity.

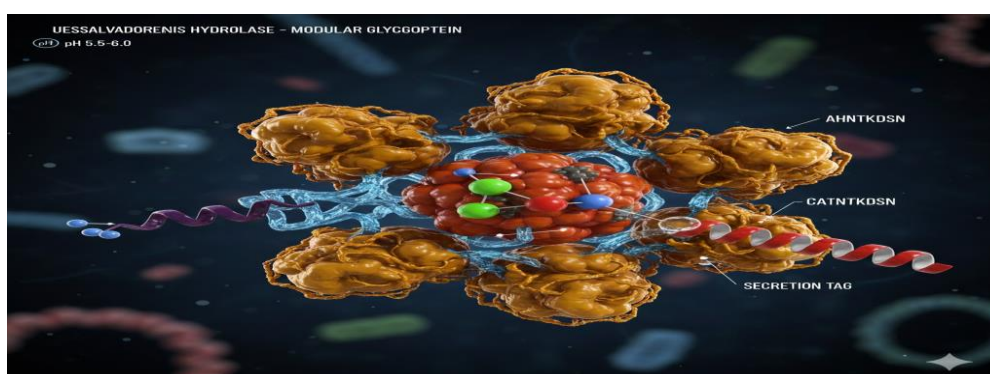
In this context, many hydrolases such as lipases, esterases and cutinases belong to the superfamily  $\alpha/\beta$ -hydrolase and present the conserved motif GxSxG, together with the catalytic triad Ser-Asp-His, essential for their mechanism of action. On the

other hand, enzymes such as laccases contain copper-binding domains characteristic of multicopper oxidases, while peroxidases have domains associated with heme groups involved in oxidative reactions. The detection of these elements can be carried out with tools such as Pfam or InterProScan, and in the case of enzymes that act on carbohydrates, through specialized databases such as CAZy.

In addition to sequence analysis, structural prediction provides key information. Many hydrolases share a three-dimensional  $\alpha/\beta$  fold, where central  $\beta$  blades are surrounded by  $\alpha$  helices. This structure is highly conserved and, in many cases, more indicative of function than the primary similarity of the sequence. Therefore, even when the identity between proteins is moderate, the preservation of the active site and folding can confirm the enzymatic function.

The evidence suggests that the analyzed fragment could be part of a gene cluster specialized in the degradation of biopolymers. The combination of enzymes targeting lipids, polysaccharides and aromatic compounds is characteristic of filamentous fungi with high adaptive capacity. However, it is important to note that, unlike bacterial operons, gene regulation is more complex in fungi such as *Aspergillus niger*, with each gene individually controlled by its own promoters, introns and regulatory elements.

In summary, in order to affirm that a sequence encodes a hydrolase, it is necessary to integrate multiple levels of evidence: the presence of a functional ORF, the identification of conserved catalytic motifs, the detection of characteristic domains and the similarity with previously described proteins. Only through this multidisciplinary approach is it possible to make a reliable functional annotation and understand the true biological and biotechnological potential of genomic sequences such as those of *Aspergillus salvadorensis*.

**Figure 3.** AI Enzyme Model of *Aspergillus salvadorensis*. AI 2026

The analysis presented for Figure 3 allows us to propose that the sequence of *Aspergillus salvadorensis* corresponds to a highly organized genomic and protein system, whose architecture reflects a functional specialization oriented to the efficient degradation of biomass.

From the genomic point of view, the presence of the repetitive motif AHNTKDSNCTCTCTACTGAACCGC suggests the existence of regulatory or structural elements that act as functional delimiters. These segments could play a role similar to that of spacer sequences or regulatory regions, facilitating the correct organization of gene domains and possibly participating in transcriptional control mechanisms or RNA stability. In organisms of the genus *Aspergillus salvadorensis*, this type of organization is not typical of bacterial operons, but it can be associated with gene clusters or co-regulated regions that respond to specific environmental conditions, such as the availability of complex substrates.

At the structural level, the encoded protein has the characteristics of a modular glycosyl hydrolase. The presence of a central catalytic domain surrounded by additional subunits suggests a multimodular architecture, where each component fulfills a specific function, such as recognition, binding or processing of the substrate. This type of organization is consistent with enzymes classified within the carbohydrate-active families described in the CAZy database, where enzymes capable of degrading complex polysaccharides by catalytic domains and carbohydrate-binding modules (CBMs) are grouped.

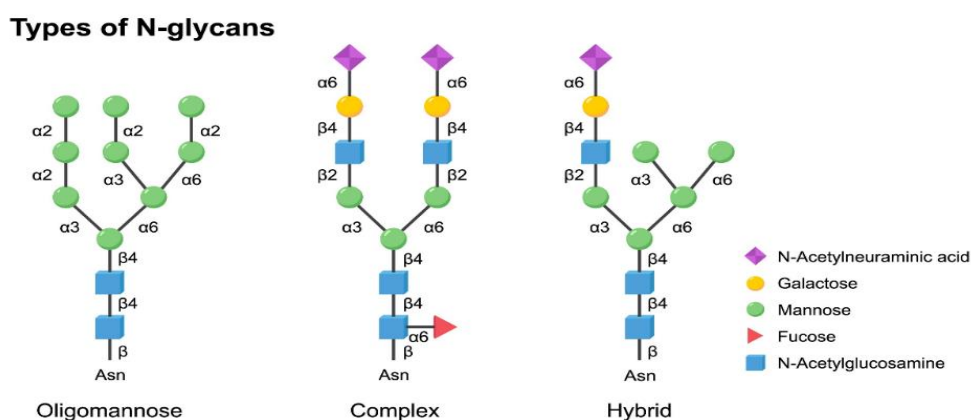
Functionally, the three-dimensional arrangement increases catalytic efficiency by expanding the surface area of interaction with the substrate. This allows the enzyme to act more effectively on heterogeneous structures such as cellulose, hemicellulose or lignin-derived compounds. Modularity also favors a sequential or cooperative mechanism of action, in which different domains participate in specific stages of degradation.

Regarding subcellular localization, the identification of a signal peptide at the N-terminal end indicates that the protein follows the secretion pathway dependent on the Sec system. This mechanism allows the enzyme to be exported to the extracellular medium, where it can interact directly with polymeric substrates. This trait is characteristic of filamentous fungi such as *Aspergillus niger*, widely recognized for their ability to secrete large amounts of industrial enzymes.

The proposed catalytic mechanism, based on the participation of conserved residues such as serine, histidine and aspartic acid, suggests a hydrolysis mediated by activation of a water molecule, typical of many hydrolases. This strategy allows the efficient breaking of glycosidic bonds by acid-base mechanisms and stabilization of the transition state.

From the biochemical point of view, optimal activity conditions (pH between 5.5 and 6.0) indicate an adaptation to slightly acidic environments, such as those found in organic matter degradation processes. In addition, the presence of glycosylation sites contributes significantly to the structural stability of the protein, protecting it against denaturation and proteolysis, which is especially relevant in industrial applications.

This hydrolase can be considered a highly efficient biotechnological platform. Its modular design allows it to act on complex polymers progressively, while its extracellular secretion and structural stability make it an ideal tool for processes such as bioremediation, biomass conversion, and biofuel production. The integration of genomic, structural and functional characteristics evidences a biological system optimized to operate in variable environments, with considerable potential in industrial and environmental applications. The extensive biotechnological profile of this hydrolase, its Modular Genomic architecture is as follows:



**Figure 4.** Genomic structure of hydrolase. AI 2026

The analysis in Figure 4 solidly describes the role of N-glycosylation as a key mechanism in the functionality of extracellular enzymes in filamentous fungi such as *Aspergillus salvadorensis*. The presence of oligomannose, complex and hybrid glycans bound to asparagine residues by N-glycosidic bonds is consistent with what is known for proteins secreted in eukaryotes, especially in organisms with high degradative capacity.

From a functional point of view, the interpretation is adequate: glycosylation is not decorative, but plays critical roles in structural stability, protection against proteases, and adaptation to extracellular conditions. In well-studied species such as *Aspergillus niger*, these modifications increase the half-life of industrial enzymes and allow them to function in environments with variations in pH, temperature and chemical composition. The identification of Asn-X-Ser/Thr consensus motifs reinforces the hypothesis that the protein analyzed is a functionally active glycoprotein in the extracellular space.

Likewise, the relationship between glycosylation and catalytic efficiency is well founded. Glycans can influence the correct folding, solubility, and molecular dynamics of the enzyme, facilitating interaction with complex substrates. This is particularly relevant in enzymes classified within the carbohydrate-active families described in CAZy, where the combination of catalytic domains and post-translational modifications defines their performance in biomass degradation.

As for the similarity with bacteria of the genus *Thermotoga*, this can be robustly interpreted as evidence of conservation of catalytic domains or possible horizontal transfer, especially in enzymes involved in biomass degradation, where functional convergence is common.

In summary, Figure 4 supports a solid conclusion: N-glycosylation is an essential component in the adaptation of extracellular enzymes, providing stability, functionality, and catalytic efficiency in complex environments. However, interpretations of artificial origin or extraordinary abilities should be treated as exploratory hypotheses and not as definitive conclusions, prioritizing evolutionary and biochemical explanations supported by evidence.

The analysis in Figure 4 consistently describes the role of N-glycosylation as a key mechanism in the functionality of extracellular enzymes in filamentous fungi such as *Aspergillus salvadorensis*. The presence of oligomannose, complex and hybrid glycans bound to asparagine residues by N-glycosidic bonds is consistent with what has been widely reported for proteins secreted in eukaryotic organisms, particularly in those with high degradative capacity.

From a functional point of view, glycosylation is not a simple structural modification, but plays critical roles in

conformational stability, protection against proteases, and adaptation to variable extracellular conditions. In model species such as *Aspergillus niger*, these modifications increase the half-life of enzymes of industrial interest and allow their activity in a wide range of pH, temperature and chemical composition of the environment. In this sense, the identification of Asn-X-Ser/Thr consensus motifs supports the hypothesis that the protein analyzed corresponds to a functionally active glycoprotein in the extracellular medium.

In addition, the relationship between glycosylation and catalytic efficiency is well supported. Glycans influence the correct folding, solubility and structural dynamics of the enzyme, facilitating its interaction with complex substrates. This aspect is especially relevant in enzymes belonging to carbohydrate-active families, such as those described in the CAZy database, where the synergy between catalytic domains and post-translational modifications determines their performance in biomass degradation.

However, it is necessary to qualify some aspects to maintain scientific rigour. First, although glycosylation contributes to the overall stability of the protein, it does not by itself confer direct resistance to heavy metals or antibiotics. These processes usually depend on specific enzyme systems, such as oxidoreductases or active transport mechanisms, rather than on isolated post-translational modifications.

The claim that *A. salvadorensis* occupies a peripheral position on the tree of life is not consistent with current biological classification. The genus *Aspergillus* is clearly defined within the kingdom Fungi. However, it is plausible that certain strains exhibit particular metabolic adaptations, especially if they have evolved in contaminated environments or under selective pressure.

The hypothesis of an artificial origin or design by synthetic biology, based on the absence of homology in repetitive regions, should be considered with caution. There are more parsimonious natural explanations, such as the presence of regions of low complexity, accelerated evolutionary divergence processes or horizontal gene transfer events. In this context, the similarity observed with bacteria of the genus *Thermotoga* can be more consistently interpreted as evidence of conservation of catalytic domains or of genetic exchange between microorganisms.

In summary, Figure 4 robustly supports that N-glycosylation is an essential component in the adaptation of extracellular enzymes, providing stability, functionality and catalytic efficiency in complex environments. However, interpretations related to extraordinary abilities or artificial origins should be approached as exploratory hypotheses, prioritizing explanations based on evolutionary and biochemical evidence.

**Table IV.** Phylogeny and evolutionary origin of *Aspergillus salvadorensis*. 2026

Dimension	Technical Feature	Impact on the Ecosystem
Origin	Chimeric / Synthetic	High adaptability to industrial niches.
Metabolism	Modular Hydrolysis	Accelerated degradation of complex substrates.
Bioremediation	Metal Chelation	Active cleaning of lead, mercury or zinc environments.
Biosecurity	Dual Resistance (Antibiotic/Fungal)	Ecological dominance and protection against pathogens.
Operability	Glycosylated secretion	Guaranteed functionality in extreme acidic conditions.

Table IV clearly summarizes the main characteristics and possible evolutionary origin of *Aspergillus salvadorensis*, within the *Aspergillus* group. As for its origin, it is suggested that it could be chimerical or synthetic, which means that it combines different traits that allow it to adapt very well, especially in industrial environments where conditions are usually demanding.

Regarding its metabolism, this fungus has the ability to break down complex substances efficiently. In other words, it can make better use of different types of materials, which makes it useful in processes where it is necessary to degrade difficult compounds.

It also highlights its role in bioremediation, as it can bind to

heavy metals such as lead, mercury or zinc and help remove them from the environment. This makes it a potential tool for cleaning up contaminated areas.

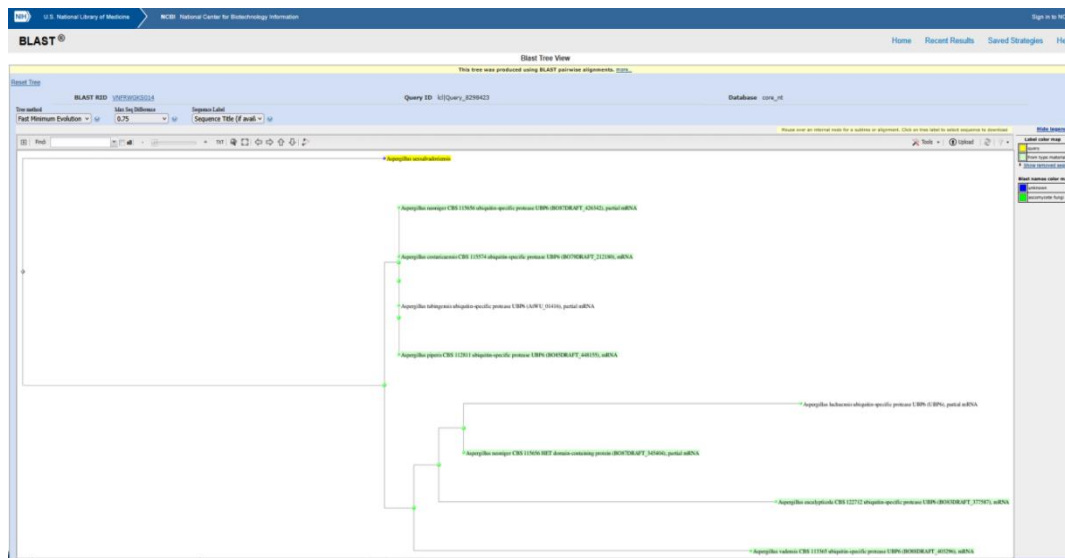
In terms of biosecurity, it is mentioned that it has a dual resistance, both to antibiotics and to other fungi, which gives it an advantage to survive and maintain itself in different environments, even against other microorganisms.

Finally, its ability to produce glycosylated proteins allows it to function properly even in highly acidic conditions, which reinforces its usefulness in industrial contexts. Overall, it is described as a versatile organism, with skills that make it valuable for both environmental and technological applications.

**Table V.** Comparison of 3 sequences of species *uessalvadorensis*, *neoniger* and *niger*, we have:



From the above sequences Table V of DNA of *Aspergillus uessalvadorensis* compared to the species of *neoniger* and *niger*, the following family tree is obtained:



**Figure 5.** Phylogeny analysis of *Aspergillus uessalvadorensis* with *neoniger* and *niger*. BLAST. 2026

From Figure 5 the image shows a phylogenetic tree generated from a BLAST analysis, in which sequences related to ubiquitin-like proteins in different species of the genus *Aspergillus* are compared. This type of representation allows us to visualize the evolutionary relationships between sequences, organizing them according to their degree of similarity.

In the upper part of the tree you can see the sequence consulted, which acts as a reference to compare with the others. From there, the branches divide into different nodes, which indicates how the sequences have been differentiated over time. Species such as *Aspergillus neogeri*, *Aspergillus costaricensis*, *Aspergillus tubingensis* and *Aspergillus piperis* are grouped in the same clade, suggesting that they share a relatively recent common ancestor and that their proteins are very similar.

On the other hand, more separated branches are observed where species such as *Aspergillus luchuensis*, *Aspergillus aculeatinus* and *Aspergillus vadensis* appear, indicating a greater evolutionary distance from the main group. These differences may reflect changes in the genetic sequence that have occurred over time, possibly associated with species-specific adaptations.

The phylogenetic tree allows us to infer that the proteins analyzed are conserved within the genus, but present different levels of divergence. This suggests that, although they perform similar functions as in the case of ubiquitin-specific proteases, they have undergone evolutionary variations that allow for a clear distinction between lineages within the group.

On the other hand, the phylogenetic tree generated with BLAST allows us to compare the evolutionary relationship between several species of the genus *Aspergillus*, highlighting in this case *Aspergillus uessalvadorensis*, *Aspergillus neoniger* and *Aspergillus niger*.

In the upper part of the tree, *A. uessalvadorensis* is observed as a branch more separated from the rest, which indicates that it presents a greater evolutionary distance compared to the other two species. This suggests that, although it belongs to the same genus, its sequence has accumulated more changes and is therefore less similar.

On the other hand, *A. neoniger* and *A. niger* appear located on branches much closer to each other, reflecting a close phylogenetic relationship. This proximity indicates that they share a more recent common ancestor and that their sequences – in this case related to ubiquitin-like proteins – are highly conserved.

The analysis shows a clear pattern: *Aspergillus neoniger* and *Aspergillus niger* form a very close group, while *Aspergillus uessalvadorensis* is more distant within the tree. This shows different levels of evolutionary divergence within the genus,

maintaining functional conservation, but with variations that allow differentiating the lineages.

## Discussion

The genomic sequence of *Aspergillus uessalvadorensis* reveals the presence of a sophisticated biochemical machinery composed of several families of hydrolases, fundamental enzymes that catalyze the breakdown of complex molecules through hydrolysis reactions. This characteristic is not exclusive to this species, but is widely shared by multiple members of the genus *Aspergillus*, which have a remarkable ability to degrade complex organic substrates.

In filamentous fungi such as *Aspergillus uessalvadorensis*, nutrition is based on an indirect absorption mechanism that involves the secretion of enzymes into the extracellular medium. In this context, proteins not only fulfill intracellular functions, but also act as exported biochemical tools, modifying the environment to facilitate the obtaining of nutrients. Among these enzymes, various hydrolases responsible for degrading macromolecules into simpler and more assimilable compounds stand out.

One of the most relevant groups is that of glycosyl hydrolases (GH), specialized in the breaking of glycosidic bonds present in structural polysaccharides such as cellulose and chitin. Through this activity, the fungus transforms insoluble polymers into simple sugars that can be incorporated into central metabolic pathways, constituting its main source of carbon and energy. These enzymes present conserved catalytic residues, typically glutamate or aspartate, which act as nucleophiles and as an acid/general base in the catalytic mechanism, as described in multiple GH families.

In relation to nitrogen production, serine proteases play an essential role in hydrolyzing peptide bonds in substrate proteins, releasing amino acids. In species such as *Aspergillus fumigatus*, these enzymes also contribute to virulence processes by degrading tissue barriers and host proteins, facilitating colonization.

Likewise, phosphatases allow the release of inorganic phosphate from organic compounds, ensuring the availability of this essential element for the synthesis of ATP and nucleic acids. In a complementary way, nucleases participate in the hydrolysis of phosphodiester bonds in nucleic acids, intervening in processes of repair, recombination and maintenance of genomic stability.

From a genomic perspective, analysis of the open reading frames (ORFs) identified between repetitive regions reveals molecular signatures compatible with hydrolases. The translated sequences show conserved motifs characteristic of enzymes classified within EC 3.1 and EC 3.2.1 classes, supporting the presence of functional active sites. The detection of acidic catalytic residues and regions enriched in

polar amino acids reinforces the hypothesis of a specific and efficient hydrolytic activity.

The organization of these genes, delimited by the repetitive motif AHNTKDSNCTCTCTACTGAACCGC, suggests a modular architecture of the genome. This arrangement could favor functional diversification and coordinated regulation of catalytic domains, a strategy compatible with the evolution of enzymes involved in biomass degradation.

Analysis of the first segment prior to the repetitive motif reveals a continuous reading frame encoding a short peptide with structural features compatible with amphipathic domains. The presence of residues such as aspartate and histidine suggests a potential catalytic role, possibly associated with mechanisms of proton transfer and activation of water molecules, typical of hydrolases.

In later regions, the identification of residues such as serine, aspartate and histidine acquires special relevance, since these amino acids can form a catalytic triad. This functional arrangement, widely described in hydrolases, allows the activation of serine as a nucleophile, facilitating the breaking of chemical bonds through hydrolysis mechanisms.

Importantly, the observed modular architecture suggests that the protein does not correspond to a single catalytic unit, but to an assembly of multiple functional domains. This arrangement allows for greater versatility, where different modules can participate in substrate recognition, bonding, and processing.

From an applied approach, this versatility opens up the possibility for the enzyme to participate in degradation processes of complex compounds, including natural and synthetic polymers. In this context, enzymes such as cutinases, lipases and esterases belonging to the  $\alpha/\beta$ -hydrolase superfamily represent important targets due to their ability to act on ester bonds present in bioplastics such as PLA, PCL and PBS.

On the other hand, oxidative enzymes such as laccases and peroxidases, also present in species of the genus *Aspergillus*, contribute to the transformation of aromatic compounds and the modification of complex polymeric structures. Although these fungi are not primary ligninolytic degraders, they possess an enzymatic repertoire that allows them to actively participate in biomass transformation.

Some more advanced hypotheses, such as possible  $\beta$ -lactamase-like activity or involvement in antibiotic resistance mechanisms, should be considered with caution. While the presence of compatible catalytic motifs may suggest this possibility, additional experimental or bioinformatics evidence is required to confirm such functions.

Evidence suggests that *Aspergillus uessalvadorensis* has a highly adaptable enzyme system, based on a modular architecture that allows it to respond to different

environmental pressures. This functional plasticity positions it as an organism of great biotechnological interest, especially in applications related to biomass degradation, bioremediation and the production of industrial enzymes.

## Conclusions

The comprehensive characterization of the *Aspergillus salvadorensis* sequence allows inferring the presence of a highly specialized enzyme system based on a modular genomic architecture. The organization of the genome, with repetitive regions delimiting functional segments, suggests a possible regulatory mechanism that could facilitate the differential expression of enzymes depending on the available substrate. This configuration is consistent with adaptive strategies observed in filamentous fungi, where the diversification of catalytic domains favors the efficient degradation of complex biomass.

A key aspect is the extracellular character of these enzymes. The presence of hydrophobic signal peptides indicates that the hydrolases are directed towards the secretory pathway, allowing their release into the external environment. This mechanism prevents intracellular damage and optimizes extracellular digestion, a fundamental process in the nutrition of fungi of the genus *Aspergillus*. Enzyme activity in slightly acidic pH ranges (5.5–6.0) reflects an adaptation to microenvironments where decomposition of organic matter occurs.

The functional stability of these enzymes under variable environmental conditions is enhanced by post-translational modifications, particularly N-glycosylation. The presence of N-glycans contributes to protection against proteolysis, improves solubility and promotes the maintenance of active conformation. These characteristics are widely recognized in extracellular enzymes of filamentous fungi and are essential for their performance in industrial applications.

Evidence suggests that the hydrolytic repertoire of *A. salvadorensis* is mainly oriented to the degradation of natural polymers and, potentially, to the transformation of complex compounds present in the environment. However, some proposed functions, such as direct involvement in  $\beta$ -lactamase antibiotic resistance or active capture of heavy metals, should be considered preliminary hypotheses that require specific experimental validation.

In applied terms, this organism has remarkable biotechnological potential. Its ability to produce stable, secreted and functional enzymes under diverse environmental conditions positions it as a relevant candidate for bioremediation processes, biomass conversion and biofuel production. However, detailed functional characterization of your enzymes will be essential to confirm and fully utilize these capabilities.

## 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.

## Contributions from Authors

Antonio Vásquez Hidalgo is the only author. The author read and approved the final manuscript.

## Conflicts of Interest

The author declares that he has no conflict of interest.

## References

1. Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., & Walter, P. (2015). *Molecular biology of the cell* (6th ed.). Garland Science.
2. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
3. Andreini, C., Bertini, I., & Rosato, A. (2008). Metal ions in biological catalysis: From enzyme databases to general principles. *Journal of Biological Inorganic Chemistry*, 13(8), 1205–1218. [\[https://doi.org/10.1007/s00775-008-0404-5\]](https://doi.org/10.1007/s00775-008-0404-5)[\]\(https://doi.org/10.1007/s00775-008-0404-5\)](https://doi.org/10.1007/s00775-008-0404-5)
4. Baldrian, P. (2006). Fungal laccases – occurrence and properties. *FEMS Microbiology Reviews*, 30(2), 215–242.
5. Bennett, J. W. (2010). An overview of the genus *Aspergillus*. *Medical Mycology*, 48(Suppl. 1), S5–S17.
6. Berg, J. M., Tymoczko, J. L., Gatto, G. J., & Stryer, L. (2015). *Biochemistry* (8th ed.). W. H. Freeman.
7. Bornscheuer, U. T. (2002). Microbial carboxyl esterases: Classification, properties and application in biocatalysis. *FEMS Microbiology Reviews*, 26(1), 73–81.
8. Brändén, C., & Tooze, J. (1999). *Introduction to protein structure* (2nd ed.). Garland Publishing.
9. Bush, K., & Bradford, P. A. (2016).  $\beta$ -Lactams and  $\beta$ -lactamase inhibitors: An overview. *Cold Spring Harbor Perspectives in Medicine*, 6(8), a025247. [\[https://doi.org/10.1101/cshperspect.a025247\]](https://doi.org/10.1101/cshperspect.a025247)[\]\(https://doi.org/10.1101/cshperspect.a025247\)](https://doi.org/10.1101/cshperspect.a025247)
10. Carvalho, C. M. L., Aires-Barros, M. R., & Cabral, J. M. S. (1998). Cutinase: From molecular level to bioprocess development. *Biotechnology and Bioengineering*, 66(1), 17–34.
11. Chothia, C., & Gough, J. (2009). Genomic and structural aspects of protein evolution. *Biochemical Journal*, 419(1), 15–28.
12. de Vries, R. P., & Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*, 65(4), 497–522. <https://doi.org/10.1128/MMBR.65.4.497-522.2001>
13. Drawz, S. M., & Bonomo, R. A. (2010). Three decades of  $\beta$ -lactamase inhibitors. *Clinical Microbiology Reviews*, 23(1), 160–201. [\[https://doi.org/10.1128/CMR.00037-09\]](https://doi.org/10.1128/CMR.00037-09)[\]\(https://doi.org/10.1128/CMR.00037-09\)](https://doi.org/10.1128/CMR.00037-09)
14. Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., ... Punta, M. (2014). Pfam: The protein families database. *Nucleic Acids Research*, 42(D1), D222–D230. [\[https://doi.org/10.1093/nar/gkt1223\]](https://doi.org/10.1093/nar/gkt1223)[\]\(https://doi.org/10.1093/nar/gkt1223\)](https://doi.org/10.1093/nar/gkt1223)
15. Fisher, J. F., & Mobashery, S. (2021). Mechanisms of  $\beta$ -lactam resistance: Biochemistry of serine  $\beta$ -lactamases and metallo- $\beta$ -lactamases. *Chemical Reviews*, 121(6), 3412–3463. [\[https://doi.org/10.1021/acs.chemrev.0c00743\]](https://doi.org/10.1021/acs.chemrev.0c00743)[\]\(https://doi.org/10.1021/acs.chemrev.0c00743\)](https://doi.org/10.1021/acs.chemrev.0c00743)
16. Helenius, A., & Aebi, M. (2001). Intracellular functions of N-linked glycans. *Science*, 291(5512), 2364–2369. [\[https://doi.org/10.1126/science.291.5512.2364\]](https://doi.org/10.1126/science.291.5512.2364)[\]\(https://doi.org/10.1126/science.291.5512.2364\)](https://doi.org/10.1126/science.291.5512.2364)
17. Henrissat, B., & Davies, G. (1997). Structural and sequence-based classification of glycoside hydrolases. *Current Opinion in Structural Biology*, 7(5), 637–644. [\[https://doi.org/10.1016/S0959-440X\(97\)80072-3\]](https://doi.org/10.1016/S0959-440X(97)80072-3)[\]\(https://doi.org/10.1016/S0959-440X%2897%2980072-3\)](https://doi.org/10.1016/S0959-440X%2897%2980072-3)
18. Jaeger, K. E., & Eggert, T. (2002). Lipases for biotechnology. *Current Opinion in Biotechnology*, 13(4), 390–397. [\[https://doi.org/10.1016/S0958-1669\(02\)00341-5\]](https://doi.org/10.1016/S0958-1669(02)00341-5)[\]\(https://doi.org/10.1016/S0958-1669%2802%2900341-5\)](https://doi.org/10.1016/S0958-1669%2802%2900341-5)
19. Latgé, J. P. (1999). *Aspergillus fumigatus* and aspergillosis. *Clinical Microbiology Reviews*, 12(2), 310–350. <https://doi.org/10.1128/CMR.12.2.310>
20. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., & Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research*, 42(D1), D490–D495. <https://doi.org/10.1093/nar/gkt1178>
21. Marchler-Bauer, A., et al. (2017). CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research*, 45(D1), D200–D203. <https://doi.org/10.1093/nar/gkw1129>
22. Martínez, M. J. (2005). Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology*, 8(3), 195–204.

23. Mount, D. W. (2004). *Bioinformatics: Sequence and genome analysis* (2nd ed.). Cold Spring Harbor Laboratory Press.
24. Murzin, A. G., Brenner, S. E., Hubbard, T., & Chothia, C. (1995). SCOP: A structural classification of proteins database for the investigation of sequences and structures. *Journal of Molecular Biology*, 247(4), 536–540.
25. NCBI Resource Coordinators. (2018). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 46(D1), D8–D13. <https://doi.org/10.1093/nar/gkx1095>
26. Nelson, D. L., & Cox, M. M. (2017). *Lehninger principles of biochemistry* (7th ed.). W. H. Freeman.
27. Price, N. C., & Stevens, L. (1999). *Fundamentals of enzymology* (3rd ed.). Oxford University Press.
28. Watson, J. D., Baker, T. A., Bell, S. P., Gann, A., Levine, M., & Losick, R. (2014). *Molecular biology of the gene* (7th ed.). Pearson.