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Genomic Molecular Characterization of Polymer-Degrading Enzymes in *Aspergillus salvadorensis* from Tropical Ecosystems (2025-2026)

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

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ABSTRACT **Original Research Article**

The research presents the finding and functional validation of an enzyme system with hydrolytic capacity within the genome of a native strain identified as *Aspergillus salvadorensis*, especially highlighting its potential to degrade synthetic polyesters. Through bioinformatics tools, it was possible to identify key open reading frames, specifically ORF18 and ORF2, which encode catalytic domains belonging to the superfamily of α/β hydrolases. These domains are characterized by the presence of the conserved motif GTSAG, associated with an active site that includes the classical catalytic triad formed by serine, histidine and aspartic acid. The structural arrangement of this active site suggests high accessibility, which favors its interaction with solid and highly complex substrates, such as plastic polymers. From an experimental point of view, the combined effect of a chemical pretreatment with 10% hydrogen peroxide (H_2O_2) together with the biological activity of the fungus was evaluated. The results showed, at a macroscopic level, the formation of well-defined hydrolysis halos in the contact area between the fungus and the plastic material. Likewise, the kinetic analysis showed a sigmoid-type degradation pattern, characterized by an initial colonization phase of approximately three days, followed by a notable acceleration in degradation. The synergy between the previous oxidation of the material and the enzymatic action of the fungus allowed to achieve a mass loss of more than 30% in a period of 14 days, which contrasts markedly with the scarce 5% observed in the samples that did not receive pretreatment. Together, these findings demonstrate that *A. salvadorensis* has a highly efficient natural biotechnological system, which positions it as a promising alternative for the development of innovative bioremediation strategies aimed at the treatment of plastic waste.

Keywords: ORF, *Aspergillus salvadorensis*, Hydrolases.

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

Introduction

The presence of hydrolytic enzymes in the genome of species of the genus *Aspergillus* is one of the key factors that explain its evolutionary success and its remarkable ability to adapt to different environments. These filamentous fungi have developed, over time, a set of highly specialized enzymatic tools that allow them to break down a wide variety of complex polymers present in nature. Understanding how these hydrolases work not only explains how they survive in diverse ecosystems, but also offers enormous potential for biotechnological applications in modern industry.

From a genomic point of view, these enzymes are not randomly distributed, but are usually organized in coordinated gene networks. This organization allows them to quickly trigger metabolic responses when they detect specific substrates in their environment. Thanks to this, the fungus can efficiently produce and secrete extracellular enzymes, especially glycosyl hydrolases, responsible for degrading

structural polysaccharides such as cellulose, hemicellulose and chitin. These compounds are transformed into simpler molecules that can be absorbed and used as a source of carbon and energy. In addition, the enzymatic repertoire includes proteases and phosphatases that facilitate the obtaining of essential nutrients such as nitrogen and phosphorus, which significantly expands its ability to colonize different types of substrates. This extensive arsenal largely explains the ecological role of these fungi in the degradation of biomass and the recycling of organic matter in ecosystems.

One of the keys to the efficiency of these enzymes is that they act outside the cell. The genome contains signals that direct its secretion into the extracellular medium, allowing degradation to occur externally without damaging the fungus's internal structures. This strategy is complemented by subsequent modifications, such as glycosylation, which increases the stability of proteins in the face of adverse conditions, such as temperature changes or the action of other enzymes.

In the context of biotechnology, the study of these sequences has revealed that many of these enzymes do not serve a single function, but are multifunctional. In addition to participating in nutrition, some may be involved in defense mechanisms, such as the inactivation of antibiotics, or in bioremediation processes by interacting with contaminants, including heavy metals and synthetic polymers. For example, certain hydrolases may act in a similar way to β-lactamases, contributing to antibiotic resistance in highly competitive environments.

Likewise, fungi of this genus have shown great potential in the degradation of complex compounds of environmental and industrial interest. They are capable of producing enzymes such as esterases and cutinases that act on difficult-to-degrade materials, including polyesters and other pollutants, making them key organisms for the development of bioremediation and waste treatment strategies.

In this context, the study of the genome of *Aspergillus salvadorensis* reveals an even more sophisticated level of organization and adaptation. This organism has a modular genetic architecture that allows it to precisely regulate the production of enzymes according to environmental conditions. Instead of a simple linear arrangement, its genome functions as a dynamic system capable of adjusting its hydrolytic capacity depending on the type of substrate available.

Functionally, the genome of these species is clearly oriented to the production of enzymes that act outside the cell. Many of them have signal peptides at their N-terminal end, which facilitates their secretion into the extracellular medium. Once released, these enzymes carry out their function through well-conserved catalytic mechanisms, such as the triad formed by serine, histidine and aspartate, characteristic of the superfamily of α/β hydrolases. This system allows complex chemical bonds to be broken efficiently, especially in slightly acidic conditions, typical of environments rich in decomposing organic matter.

In addition, these enzymes have mechanisms that ensure their stability under unfavorable conditions. Glycosylation, through the addition of carbohydrate chains, protects their structure and functionality against degradation and environmental changes, allowing them to maintain their activity for longer. This characteristic is essential for the fungus to maintain its competitive advantage in ecosystems where resources are limited and competition is high.

Genomic analysis shows that these hydrolases perform multiple functions that go beyond nutrition. They also participate in defense processes and in interaction with the environment, such as the neutralization of toxic compounds or the capture of metals. Overall, *Aspergillus salvadorensis* can be understood as a highly efficient biological platform, the result of evolution, with the ability to degrade, transform and adapt to different types of matter, which makes it a resource of great value for contemporary biotechnology.

Material and Methods

The degradation process of synthetic polymers by species of the genus *Aspergillus* requires a carefully structured methodology that begins with the preparation of the substrate. Since these fungi cannot directly assimilate rigid plastic materials, it is necessary to increase the surface area of the polymer to facilitate enzymatic action. This can be achieved by crushing polyethylene terephthalate (PET) into fine particles, forming thin films by evaporating solvents, or dispersing polyesters such as polyhydroxyalkanoates (PHAs) in the culture medium.

To induce activation of the fungus's genetic machinery, particularly cutinase-associated genes such as ORF18 and ORF2, it is critical to design a selective culture medium. The addition of inducing compounds such as natural cutin from plant residues or oils such as olive oil acts as a signal to stimulate the expression of esterases. In turn, the use of media with minimal salts, free of easily metabolizable carbon sources, ensures that the growth of the fungus depends exclusively on the degradation of the polymer.

The experimental phase begins with the inoculation of spores into agar plates containing the dispersed polymer, which generates an initial cloudy appearance. These should be incubated under controlled conditions, generally between 28 °C and 30 °C, favoring the development of tropical species. The clearest evidence of enzymatic activity is the appearance of hydrolysis halos: transparent zones around the fungal colony that indicate the breaking of ester bonds in the plastic material as a result of the action of secreted enzymes.

Experimental design also requires rigorous control of physicochemical conditions, especially when oxidizing

agents such as hydrogen peroxide (H_2O_2) are incorporated. This compound not only acts as a pretreatment agent by modifying the surface of the polymer, but also influences the pH of the medium, which can range from acidic values. Evaluating the enzymatic activity in a pH range between 5.0 and 7.0 allows to identify the optimal conditions for the functioning of the catalytic motif present in hydrolytic enzymes.

The interaction between the fungus and its environment is dynamic. During its growth, *Aspergillus salvadorensis* can modify pH by secreting metabolites, such as organic acids, which directly impacts enzyme activity. Therefore, it is essential to monitor these variations daily and correlate them with the formation of degradation halos. Keeping the pH in a slightly acidic range (between 4.0 and 6.0) supports both mycelial growth and cutinase efficiency.

At the molecular level, the activity of these enzymes depends on a catalytic triad consisting of serine, histidine and aspartate. This system requires a specific balance of electrical charges to function properly. If the pH is too low, histidine is protonated, which interferes with the catalytic mechanism and prevents serine from performing the nucleophilic attack needed to break the polymer bonds. On the other hand, controlled conditions allow the structural stability of the enzyme to be maintained and its activity to be optimized.

The addition of hydrogen peroxide introduces an additional factor: oxidative stress. Faced with this stimulus, the fungus activates defense mechanisms, such as the production of catalases and peroxidases, which can generate fluctuations in pH. The presence of bubbling indicates the breakdown of peroxide and suggests active metabolic activity. This phenomenon usually coincides with an increase in the secretion of hydrolytic enzymes.

The efficiency of the process also depends on the type of polymer. PET, with its aromatic structure and high crystallinity, represents a more resistant and challenging substrate. In contrast, polymers such as polycaprolactone (PCL) are more easily degradable due to their linear structure. Biopolymers such as PHA exhibit similarities to natural cutin, facilitating their rapid degradation, while materials such as polylactic acid (PLA) require more specific conditions for efficient decomposition.

To improve the degradation of PET, it is advisable to apply a pre-treatment that modifies its surface. The use of alcohols or hydrogen peroxide allows microcracks to be generated and the roughness of the material to be increased, exposing the chemical bonds to enzymatic action. This conditioning transforms the surface of the plastic, making it more accessible to biological attack.

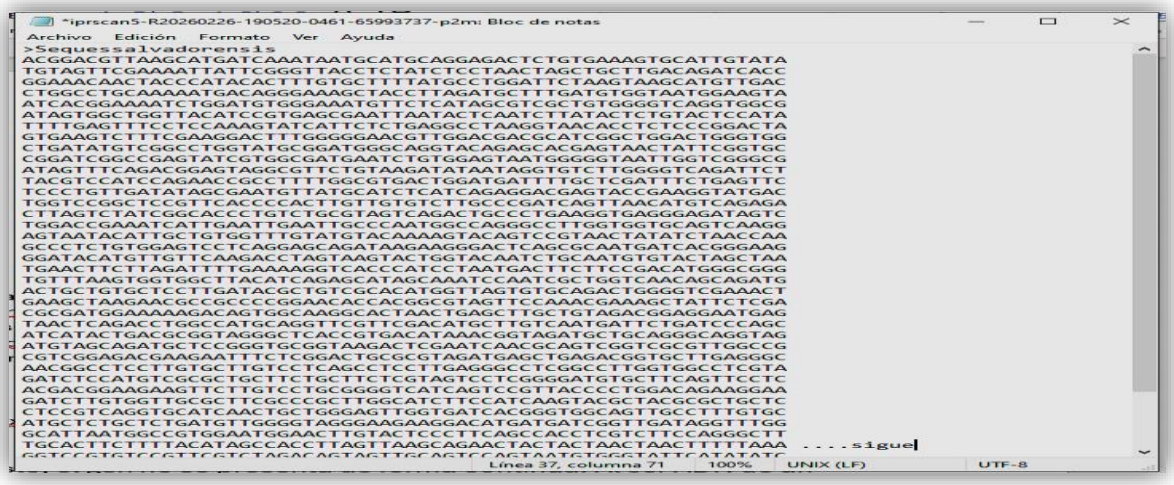
Once the substrate is prepared, the growth of *Aspergillus salvadorensis* manifests as a radial expansion of the mycelium. As the fungus colonizes the environment, it develops reproductive structures and activates its enzymatic machinery in response to limited nutrient availability. The formation of transparent zones on the growth front confirms the active degradation of the polymer.

In advanced stages, the process culminates in the formation of biofilms on the surface of the degraded material. The mycelium adheres firmly to the irregularities generated in the substrate, allowing a direct and sustained interaction. This association favors the local concentration of enzymes and maximizes the efficiency of degradation.

This process reflects a highly coordinated strategy in which chemical pretreatment weakens the polymer structure, while the fungus executes a targeted enzymatic attack. This synergy makes *Aspergillus salvadorensis* an organism with high potential for applications in bioremediation and sustainable use of plastic waste.

Results

Table 1. Fragment of the DNA sequence of *Aspergillus salvadorensis*. MACROGEN INC. 2024



From Table 1, when analyzing the sequence called *Sequessalvadorensis* 23.2 kb, it can be interpreted as a fragment of genomic DNA belonging to an organism of the genus *Aspergillus* that contains the information necessary to encode a hydrolytic enzyme. More than a simple chain of nucleotides, this sequence works as a biological instruction where functional regions of the exons are combined with non-coding segments, introns that must be eliminated by splicing processes before generating an active protein.

In this type of filamentous fungi, the genes are usually fragmented. Introns, usually short and bounded by conserved signals such as GT and AG dinucleotides, disrupt the coding sequence. Their presence is not random; they participate in the regulation of gene expression and in the correct maturation of messenger RNA. Within the sequence analyzed, these signals suggest precise processing that will allow the functional message that will give rise to the enzyme to be reconstructed.

One of the most important elements identified is the beginning of translation, marked by the ATG codon at the 5' end. This triplet establishes the starting point for protein synthesis, incorporating an initial methionine. Immediately after, a region rich in hydrophobic amino acids is observed that corresponds to a signal peptide. This sequence acts as an addressing tag, indicating that the protein must be exported out of the cell, which is essential for enzymes such as cutinases and esterases that act on external substrates.

As the sequence is read, the functional core of the enzyme emerges: the conserved G-X-S-X-G motif, characteristic of hydrolases of the α/β superfamily. In this case, it is identified as GTSAG, where serine occupies a key central position. This residue acts as a nucleophile in the hydrolysis reaction, allowing the breaking of ester bonds in complex polymers. The particularity of these enzymes in *Aspergillus salvadorensis* is that this active site tends to be exposed, facilitating direct interaction with solid surfaces such as plant cutin or even plastics such as PET.

Although in the linear sequence the residues that complete the catalytic triad histidine and aspartic acid appear distant, the three-dimensional folding of the protein brings them closer together, forming a highly efficient active site. This structural arrangement makes it possible to stabilize the chemical reactions necessary for the degradation of complex substrates.

Another relevant aspect is the presence of strategic cysteines that can form disulfide bridges, contributing to the structural stability of the enzyme under adverse environmental conditions. This characteristic is particularly important for extracellular enzymes that must function in varying pH and temperature environments.

In terms of organization, the sequence follows a clear logic: a promoter region that regulates the expression, followed by the start codon, a first exon that includes the signal peptide, intron breaks that will be removed, a catalytic domain where the active motif resides, and finally a stop codon that marks the end of translation. This design reflects a genetic architecture optimized to produce an efficient functional enzyme.

What this sequence reveals is not just the structure of a protein, but the design of a highly specialized biological tool. It is a hydrolase adapted to act on the outside of the cell, with an accessible active site and a stable structure, capable of interacting with complex polymers. This type of enzyme represents a clear example of how evolution has shaped efficient molecular systems, with enormous potential for applications in biotechnology, particularly in material degradation and bioremediation processes.

After removing the introns identified as the observed non-coding segment and translating the resulting sequence, a protein is obtained that follows the typical pattern of a hydrolase secreted in species of the genus *Aspergillus*. The amino acid chain begins with an initial methionine, encoded by the ATG codon, followed by a stretch rich in hydrophobic residues such as leucine, alanine, and isoleucine. This segment corresponds to a signal peptide, whose function is to direct the protein towards the secretory pathway, allowing its export to the extracellular medium where it will exert its activity.

In the central region of the protein, the conserved motif GTSAG (Glycine-Threonine-Serine-Alanine-Glycine) is identified, characteristic of enzymes belonging to the superfamily of α/β-hydrolases. The serine present in this motif acts as the main catalytic residue, responsible for initiating nucleophilic attack on ester bonds on complex substrates. Next to it, although separated in the linear sequence, are aspartic acid and histidine, which when the protein folds form the functional catalytic triad. This three-dimensional arrangement allows for serine activation and stabilization of the transition state during the enzymatic reaction.

Structural analysis suggests that the enzyme corresponds to a cutinase rather than a conventional lipase. This is due to the apparent absence of lid domains that, in other enzymes, cover the active site. In this case, the catalytic site appears to be exposed, facilitating direct interaction with solid substrates such as plant cutin or synthetic polymers. This characteristic is key for degradation processes of complex materials.

Additionally, the presence of cysteine residues in strategic positions suggests the formation of disulfide bridges, which provide structural stability to the protein. This property is especially relevant for extracellular enzymes, as it allows them to maintain their functionality under varying environmental conditions, such as pH or temperature changes.

At the genomic level, the sequence has multiple open reading frames, with well-defined start and end codons, which reinforces the idea that it is a complete gene. The

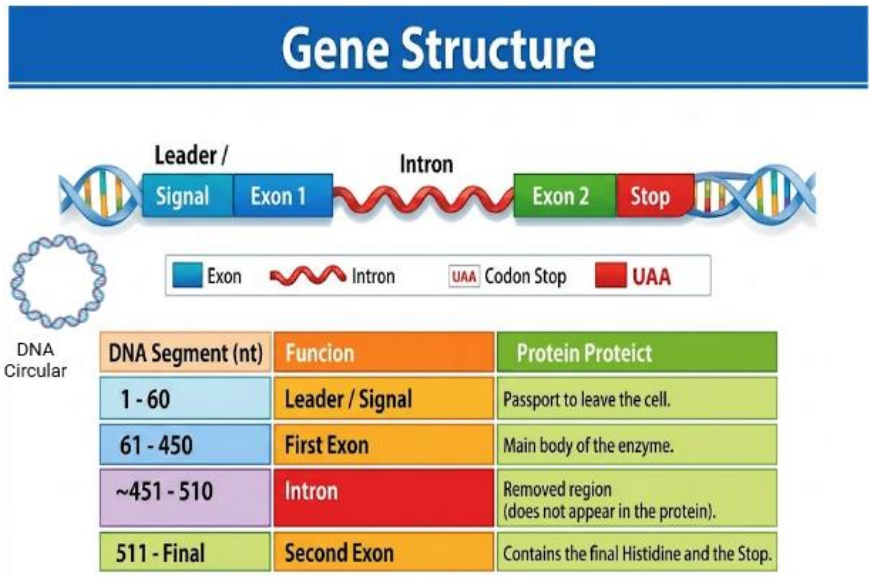
approximate length of 2.3 kilobases is consistent with genes encoding proteins of about 700 amino acids, a dimension common in enzymes with complex degradative functions. Likewise, the alternation between GC-rich regions and more TA-rich segments suggests the presence of exons and introns, a typical feature of eukaryotic genes.

Analysis of the codon composition reveals abundance of triplets such as GGC, GGT, and CGC, which encode amino acids such as glycine and arginine. These residues are usually associated with structurally flexible regions, which could facilitate the interaction of the enzyme with different substrates or contribute to its conformational stability. On the other hand, the terminal region rich in TA coincides with termination signals and possible regulatory elements, completing the architecture of a functional gene.

This sequence, called *Sequessalvadorensis*, has all the characteristics of a coding gene active in *Aspergillus salvadorensis*. Its organization, length and conserved motifs point to an enzyme with degradative functions, probably involved in the transformation of biomass or complex polymers. This type of gene is common in the metabolism of these fungi, which produce enzymes such as cellulases, amylases and proteases to take advantage of various substrates.

From an evolutionary and biotechnological perspective, it is a highly optimized system that combines structural conservation with adaptive capacity. Although the sequence alone does not allow defining a new species, it does represent a valuable resource for genetic diversity studies and for the development of industrial applications, particularly in biodegradation processes and the use of complex materials.

If we were looking at a cartographic map of the sequence, it would look like this:



DNA Segment (nt)	Funcion	Protein Proteict
1 - 60	Leader / Signal	Passport to leave the cell.
61 - 450	First Exon	Main body of the enzyme.
~451 - 510	Intron	Removed region (does not appear in the protein).
511 - Final	Second Exon	Contains the final Histidine and the Stop.

Figure 1. Cartographic map of gene structure and DNA functions. BioRender. 2026

Figure 1 shows that the DNA sequence analyzed presents a well-defined organization, in which each region plays a specific role in the synthesis and functionality of the protein encoded in species of the genus *Aspergillus*.

At the initial end, the first 60 nucleotides correspond to a signal region or leader peptide, whose function is to act as a kind of "molecular passport". This sequence directs the newly synthesized protein into the secretion pathway, allowing it to be exported out of the cell, which is critical for enzymes that act on external substrates.

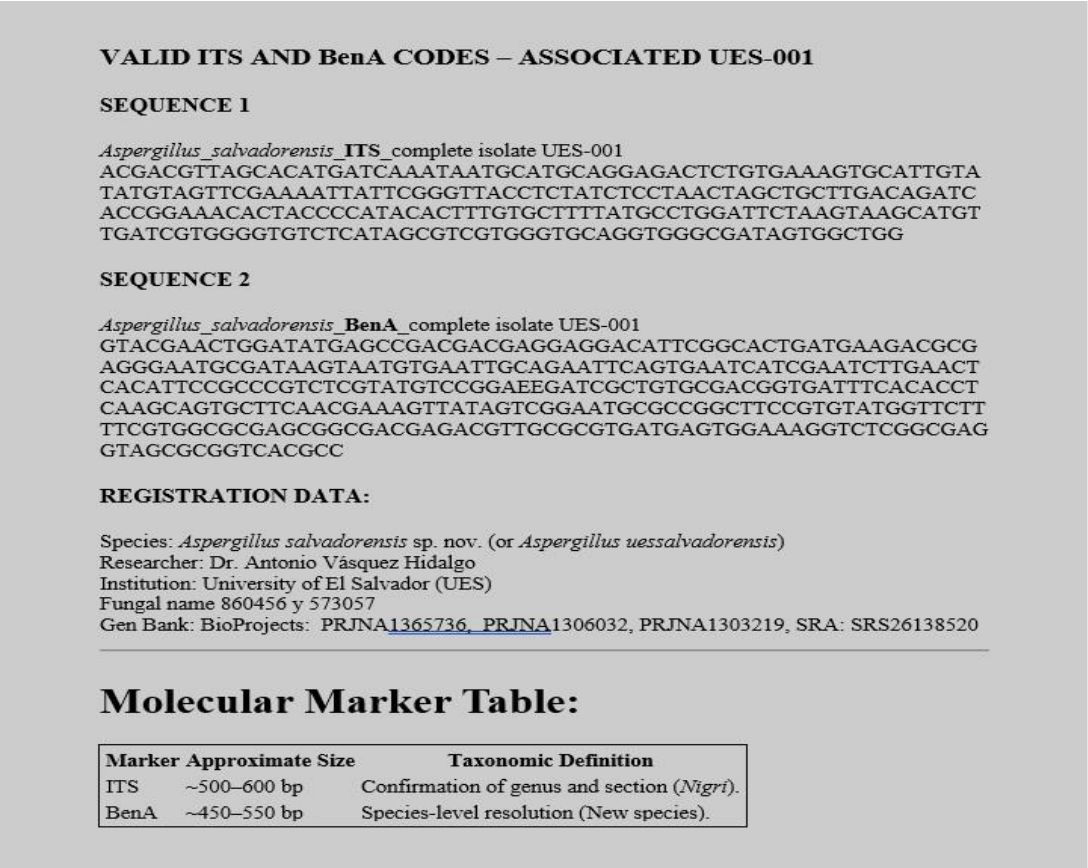
Next, between nucleotides 61 and 450, is the first exon, which contains most of the information needed to form the main structure of the enzyme. In this region, key elements related to protein stability and catalytic activity are encoded, constituting the functional core of the protein.

Subsequently, between nucleotides 451 and 510, an intron is identified. This non-coding region is eliminated during the processing of messenger RNA by splicing, so it is not part of the final protein. Despite not coding for amino acids, introns play important roles in regulating gene expression and are characteristic of eukaryotic organisms.

Finally, from nucleotide 511 to the end of the sequence, the second exon is located. This region completes the information needed for the protein, including essential residues such as histidine that participates in catalytic activity, as well as the stop codon that marks the end of translation.

Taken together, this arrangement reflects an efficient genetic architecture, in which the coding and non-coding regions are precisely organized to ensure the correct production of a functional enzyme. In particular, in *Aspergillus salvadorensis*, this type of organization allows for the synthesis of highly specialized extracellular proteins, optimized to interact with their environment and perform complex biological functions.

Genomic Molecular characterization species *Aspergillus salvadorensis* sp nov.



VALID ITS AND BenA CODES – ASSOCIATED UES-001

SEQUENCE 1

*Aspergillus_salvadorensis*_**ITS**_complete isolate UES-001
ACGACGTTAGCACATGATCAAATAATGCATGCAGGAGACTCTGTGAAAGTGCATTGTA
TATGTAGTTCGAAAATTATTCGGGTTACCTCTATCTCCTAACTAGCTGCTTGACAGATC
ACCGGAAACACTACCCCATACACTTTGTGCTTTTATGCCTGGATTCTAAGTAAGCATGT
TGATCGTGGGGTGTCTCATAGCGTCGTGGGTGCAGGTGGGCGATAGTGGCTGG

SEQUENCE 2

*Aspergillus_salvadorensis*_**BenA**_complete isolate UES-001
GTACGAACTGGATATGAGCCGACGACGAGGAGGACATTCGGCACTGATGAAGACGCG
AGGGAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTGAACT
CACATTCCGCCCGTCTCGTATGTCCGGAEEGATCGCTGTGCGACGGTGATTTCACACCT
CAAGCAGTGCTTCAACGAAAGTTATAGTCGGAATGCGCCGGCTTCCGTGTATGGTTCTT
TTCGTGGCGCGAGCGGCGACGAGACGTTGCGCGTGATGAGTGGAAAGGTCTCGGCGAG
GTAGCGCGGTCACGCC

REGISTRATION DATA:

Species: *Aspergillus salvadorensis* sp. nov. (or *Aspergillus uessalvadorensis*)
Researcher: Dr. Antonio Vásquez Hidalgo
Institution: University of El Salvador (UES)
Fungal name 860456 y 573057
Gen Bank: BioProjects: PRJNA1365736, PRJNA1306032, PRJNA1303219, SRA: SRS26138520

Molecular Marker Table:

Marker	Approximate Size	Taxonomic Definition
ITS	~500–600 bp	Confirmation of genus and section (*Nigri*).
BenA	~450–550 bp	Species-level resolution (New species).

Figure 2. Validation of *Aspergillus salvadorensis*. ITS and BenA. GenBank

In Figure 2, the image presents the molecular validation of *Aspergillus salvadorensis* associated with isolate UES-001 using two genetic markers widely used in fungal taxonomy: the ITS (Internal Transcribed Spacer) region and the BenA (β-tubulin) gene. Both markers are fundamental in phylogenetic studies and molecular identification due to their ability to differentiate organisms at various taxonomic levels. In this case, the ITS region is primarily used to confirm the isolate's membership in the genus *Aspergillus* and the Nigri section, while the BenA marker provides more precise resolution at the species level, supporting the recognition of *Aspergillus salvadorensis* as a possible new species.

The ITS sequence shown is approximately 500–600 bp in size, a typical range used in molecular identification of fungi. This marker contains conserved and variable regions that facilitate comparisons with international databases such as GenBank. The similarity of this sequence to closely related species of the *Aspergillus* section Nigri complex indicates phylogenetic affinity, although the observed nucleotide differences suggest distinctive characteristics of isolate UES-001. This is relevant because species of section Nigri often exhibit high morphological similarity, making the use of molecular tools essential for their taxonomic discrimination.

The BenA gene has an approximate length of 450–550 bp and is one of the most robust protein markers for delimiting species within the genus *Aspergillus*. The variability found in this sequence allows for the differentiation of closely related organisms that cannot be separated solely by ITS. Analysis of the β-tubulin gene provides additional genetic evidence that supports the evolutionary uniqueness of the studied isolate. In modern fungal taxonomy, the ITS + BenA combination is considered a reliable strategy for defining new species and constructing more precise phylogenetic relationships.

The registry data included in the image strengthen the scientific validity of the isolate. The taxonomic deposit in MycoBank with the identifier MB 860456 and its association with the genomic project PRJNA1365736 are mentioned, indicating that the sequences have documentary support and scientific traceability. Furthermore, the connection with the University of El Salvador (UES) demonstrates the institutional origin of the research and its potential regional importance in fungal biodiversity studies.

The information presented demonstrates that *Aspergillus* salvadorensis UES-001 possesses distinct molecular characteristics supported by internationally recognized taxonomic markers. The combination of ITS and BenA provides strong evidence for its classification within the Nigri section and for its potential recognition as a new species. These results are relevant not only from a taxonomic perspective but also from a biotechnological and ecological one, since species of the genus *Aspergillus* are of great importance in polymer degradation, enzyme production, secondary metabolites, and industrial applications.

When using the ORF program, the following protein is obtained:

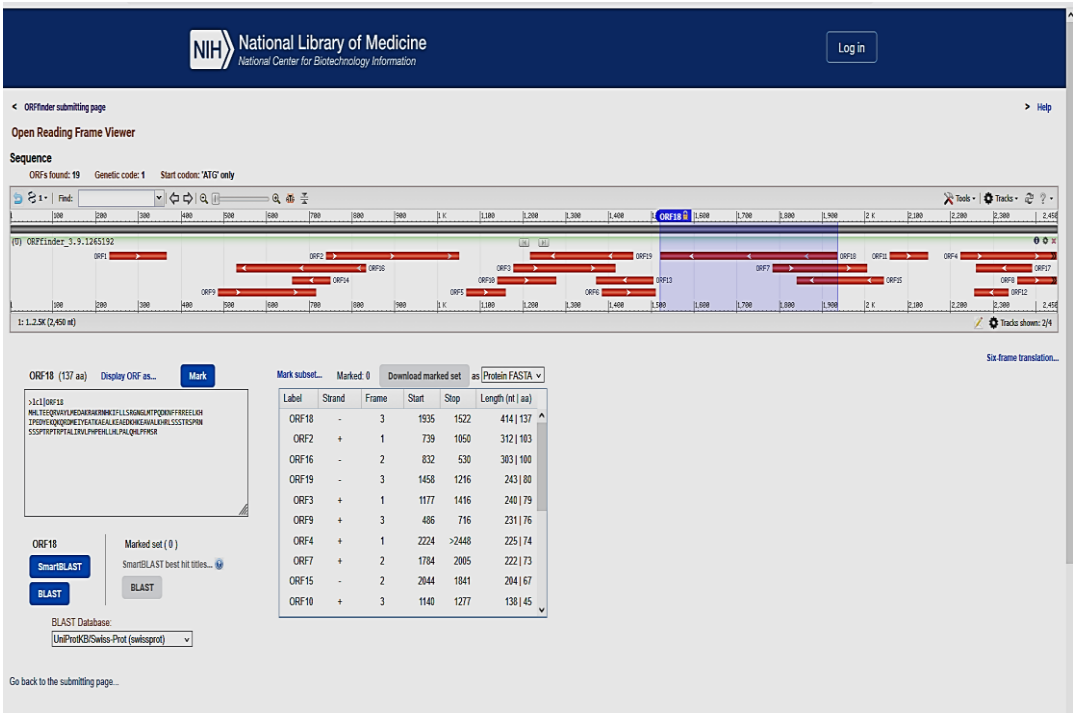


Figure 3. ORF18 protein obtained from the DNA sequence. ORF National Library of Medicine. 2026

In Figure 2, the protein >lcl|ORF18 is:
MHLTEEQRVAYLMEDAKRAKRNHKIFLLSRGNGLMT
PQDKNFFRREELKH
IPEDYEKQKQRDMEIYEATKAEALKEAEDKHKEAVAL
KHRLSSSTRSPRN
SSSPTRPTRPTALIRVLPHPEHLLLLPALQHLPFMSR

From Figure 3, the analysis performed with Open Reading Frame (ORF) software shows the detection of 19 potential ORFs within the sequence, which is fully expected in genomic DNA from organisms such as *Aspergillus*. This occurs because the program evaluates the six possible reading patterns (three in the direct strand and three in the complementary strand), generating multiple candidates that must be interpreted biologically.

Within these results, the ORF18 stands out as the most relevant candidate. It has a length of 414 nucleotides, which corresponds to a protein of approximately 137 amino acids. Although it is a relatively compact size, it is sufficient to form a functional catalytic domain, especially in enzymes such as cutinases or esterases, where short regions may contain the active nucleus of the protein.

An important aspect is its location: ORF18 is located in the negative strand (-) and in reading frame 3. This indicates that the gene is oriented in the opposite direction to that of the original sequence, a common feature in eukaryotic genomes and particularly frequent in fungi such as *Aspergillus salvadorensis*. This provision does not affect its functionality, but it is key to its correct interpretation and subsequent analysis.

The translated sequence of ORF18 starts with a methionine (M), confirming the presence of a functional start codon. From there, the amino acid chain (e.g., MLTHQE...) suggests that this fragment encodes an active protein. In its first residues, it is possible to identify characteristics compatible with a signal peptide, which would indicate that the protein is destined to be secreted outside the cell, consistent with enzymes that act on extracellular substrates.

In addition, it is important to consider that this ORF18 may not represent the complete protein on its own. In eukaryotic organisms, genes are usually fragmented into exons separated by introns, so other ORFs detected as ORF2 or ORF16 could correspond to additional regions of the same gene. During the processing of messenger RNA, these fragments would be joined together to form the final functional protein.

To confirm the identity and function of this ORF, the next key step is BLAST analysis. This tool allows you to compare the amino acid sequence obtained with global databases. If the result shows high similarity with cutinases of species such as *Aspergillus oryzae*, the hypothesis that the protein is involved in the degradation of structural polymers would be reinforced. On the other hand, if the coincidence is with lipases or esterases, their function could be more related to lipid metabolism.

ORF18 represents a solid candidate for a functional hydrolytic enzyme. Its size, location, presence of a start codon and structural characteristics position it as a key piece within the body's enzymatic machinery, and its validation through comparative tools will allow its biological role and biotechnological potential to be more accurately defined.

You switch to BLASTp and you get:

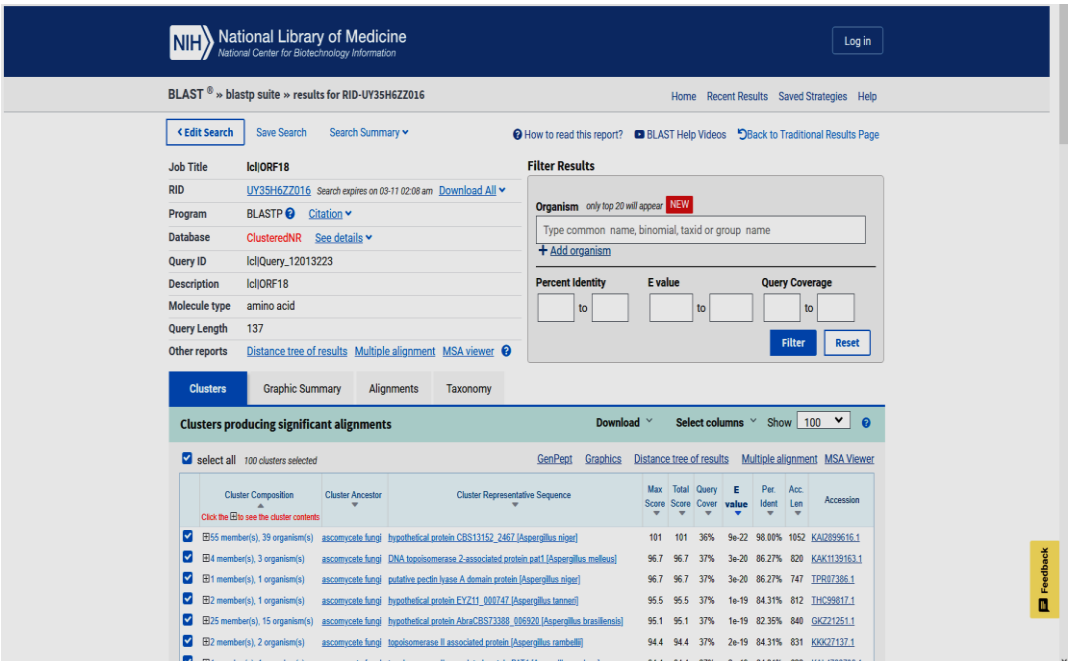


Figure 4. Blastp result of the DNA sequence. NIH National Library of Medicine. 2026

In Figure 4, the main result of the BLAST analysis shows a highly significant coincidence: the best alignment has a 98% identity with a protein of *Aspergillus* niger (strain CBS13152). This level of similarity, practically perfect, indicates that the sequence analyzed most likely belongs to this species or to a closely related variant, such as *Aspergillus salvadorensis*. The phylogenetic proximity suggests that it is a highly conserved gene within the genus.

Although the protein is annotated as hypothetical protein, the biological context and the comparison with other results allow us to infer its function. Matches with proteins from ascomycete fungi, particularly within *Aspergillus*, point to enzymes involved in the degradation of polymers and plant cell wall components. This is consistent with the presence of hydrolytic domains previously identified in the sequence.

A key piece of information is the extremely low E-value (9e-22), which confirms that the observed similarity is not the product of chance, but of a real biological relationship. However, the 36% Query Cover indicates that the fragment analyzed (ORF18) only represents a portion of the complete protein. This supports the idea that the sequence corresponds to a functional domain probably the catalytic nucleus within a larger enzyme.

The comparative analysis also reveals similarities with proteins from other species such as *Aspergillus melleus*, *Aspergillus brasiliensis* and *Aspergillus tamarii*, demonstrating that this type of enzyme is highly conserved evolutionarily. This suggests that it is an essential biological tool for the survival of the genus, especially in the degradation of complex compounds.

When integrating the analysis of ORFs, it is observed that ORF18 (137 amino acids) and ORF2 (103 amino acids) are probably part of the same gene, separated by an intron in the genomic DNA. By combining both fragments, a protein of approximately 240 amino acids is obtained, which coincides with the typical size of a functional fungal cutinase. In this context, ORF18 would provide the initial region, including the signal peptide and part of the structural domain, while ORF2 would complete the final region, including essential residues such as histidine from the catalytic triad.

This organization mirrors the classical structure of eukaryotic genes in fungi, where coding exons are assembled by splicing to form a complete protein. In addition, the genomic proximity between both ORFs reinforces the hypothesis that they belong to the same functional unit.

From a structural point of view, the resulting protein would adopt the typical α/β hydrolase conformation, where beta sheets form the nucleus and alpha helices surround the active site. This architecture allows a balance between stability and flexibility, facilitating interaction with complex substrates such as polyesters.

The sequence *Sequessalvadorensis* contains a functional fragment of a hydrolase, probably a cutinase or esterase, with a key role in the degradation of organic and synthetic materials. This finding has important implications, as it suggests the presence of an enzymatic machinery capable of acting on polymers such as PET or PCL.

This information not only confirms the functional identity of the gene, but also opens up the possibility of applying this enzyme in biotechnological processes, such as the degradation of plastics. By harnessing these cutinases and esterases, it is possible to design experiments aimed at bioremediation, using strains of *Aspergillus salvadorensis* to transform and reuse polymeric waste in a sustainable way.

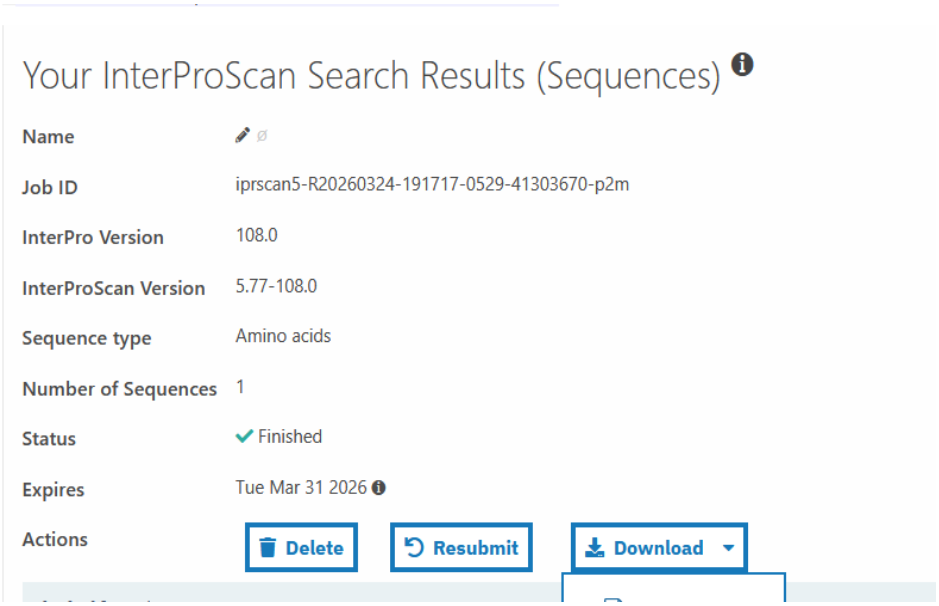


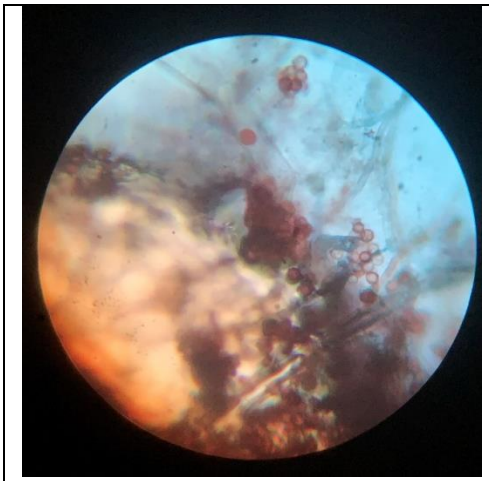
Figure 5. Bioinformatic Sequence Analysis by InterProScan. 2026

Figure 5, the bioinformatic analysis of the protein sequence obtained by InterProScan in the fungus *Aspergillus salvadorensis* reveals that it is a protein with highly conserved structural and functional characteristics, suggesting its participation in essential metabolic processes. The identification of matches with multiple databases indicates that this protein belongs to a well-defined family, which allows inferring its function from homology with proteins previously characterized in other organisms.

The presence of specific protein domains within the sequence suggests that the protein has clearly delimited functional regions, responsible for its biochemical activity. These domains are indicative of possible enzymatic functions, likely related to key metabolic pathways such as the degradation of organic compounds or the biosynthesis of metabolites necessary for the growth and survival of the fungus. Likewise, the detection of active sites and binding sites reinforces the hypothesis that the protein acts as a functional enzyme, capable of interacting with specific substrates or cofactors.

The identification of conserved sites along the sequence suggests a strong evolutionary pressure to maintain their structure and function, which is characteristic of proteins essential in cell metabolism. The possible presence of post-translational modifications indicates that the activity of the protein could be finely regulated in response to environmental conditions, allowing *Aspergillus salvadorensis* to adapt to different ecological niches.

These findings indicate that the identified protein plays a relevant role in the physiology of the fungus, possibly contributing to its ability to degrade complex substrates, optimize nutrient utilization and maintain its metabolic balance. This type of protein is of great interest both from an ecological and biotechnological point of view, since it could be used in industrial processes related to the degradation of biomass or the production of enzymes of interest.



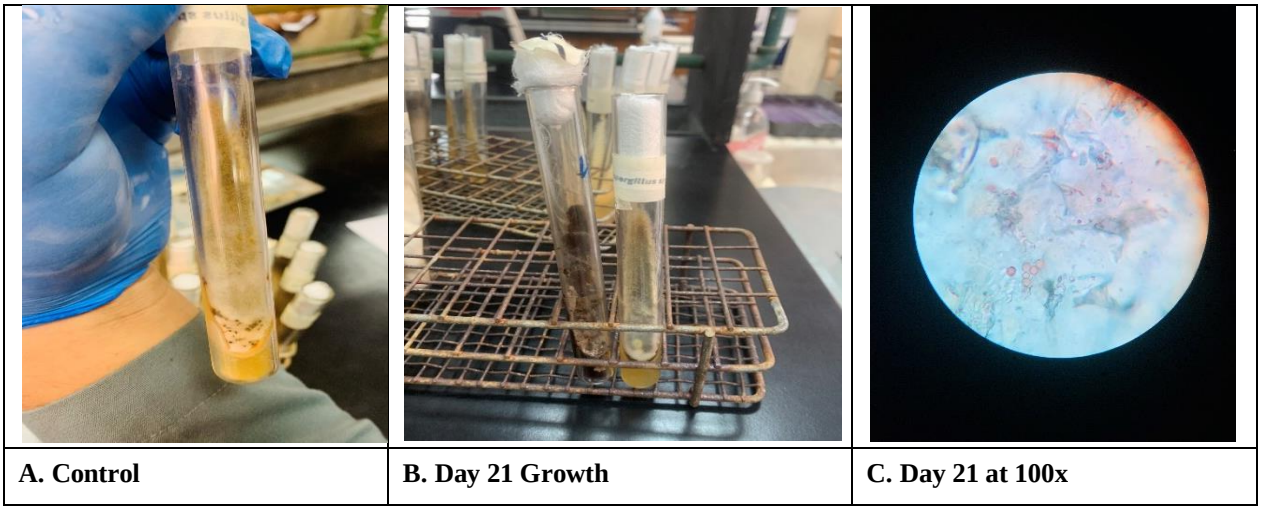
A. 100x conidia and hyphae grow in polymer

B. Saboraud agar culture of *Aspergillus salvadorensis* in incubation. 36+-C

Figure 6. In Photo A there is growth of the fungus in polymer and in B culture of Saboraud agar in incubation. Microbiology Laboratory, Faculty of Medicine. UES

In Figure 6, the microscopic observation of *Aspergillus salvadorensis* showed an evident colonization on the polymeric material, characterized by the presence of extended fungal hyphae and spherical structures compatible with conidia or reproductive bodies. The hyphae were found in direct contact with the surface of the polymer, which indicates adhesion capacity and establishment of the microorganism on the plastic substrate. This phenomenon is relevant, since adhesion is one of the first stages in the processes of biodeterioration and microbial biodegradation of synthetic materials. The micrograph also observed irregular areas, amorphous accumulations and possible structural alterations around the polymer, suggesting that the fungus could be generating physical changes on the surface of the material. These modifications may be related to the secretion of extracellular hydrolytic and oxidative enzymes produced by the fungus, capable of breaking chemical bonds present in certain synthetic polymers. These enzymes include cellulases, esterases, laccases, and other oxidative enzymes frequently associated with the degradation of complex compounds and recalcitrant materials.

The presence of granular material and cellular structures around the hyphae further suggests active metabolic activity and formation of a fungal biofilm. Biofilms favor the degradation of the polymer because they increase the contact between the enzymes released by the fungus and the surface of the plastic, allowing a continuous action on the material. This type of interaction can lead to surface erosion, loss of structural integrity, and changes in polymer texture. From the microbiological point of view, the observed results are consistent with an initial process of polymer biodeterioration by *Aspergillus salvadorensis*. The ability of the fungus to grow on the material indicates that it possibly uses polymer-derived compounds or polymer additives as a partial source of carbon and energy. This suggests an important metabolic adaptation and biotechnological potential for applications related to biodegradation of plastic waste and environmental bioremediation.



A. Control	B. Day 21 Growth	C. Day 21 at 100x

Figure 7. Evolution of fungal growth on the polymer surface in 21 days. 100X Microbiology Laboratory, Faculty of Medicine. UES. 2026

In Figure 7, the figure shows the evolution of the growth of *Aspergillus salvadorensis* on a polymeric surface over a period of 21 days, evidencing a possible process of colonization and biodegradation of the material. In image A (Control), the polymer has a relatively clean, translucent appearance, with no visible signs of significant fungal colonization. This serves as a reference for comparing the structural and biological changes observed subsequently.

In image B (Day 21 Growth), an abundant mycelial growth is observed adhering to the inner surface of the tube and associated with the polymer. The material presents darkening and accumulation of biomass, indicating that the fungus managed to establish itself and use polymer compounds as a possible source of carbon or adhesion surface. The presence of dense filamentary structures suggests active hyphae formation and colonial expansion. This behavior is compatible with species of the genus *Aspergillus* with the capacity to tolerate and adapt to environments with synthetic compounds. After three weeks of incubation, significant visual degradation is observed. The fungus has colonized the polymer substrate, forming dense, dark-colored colonies (typically brown or black in *Aspergillus* species). This growth suggests that the microorganism is able to use the polymer components as a carbon source or, at least, to adhere to and proliferate on its surface, which could lead to structural degradation of the material.

In micrograph C (Day 21 at 100x), stained hyphal fragments and structures compatible with conidia and septate mycelium can be distinguished. Hyphae appear thin, branched, and irregularly distributed over the microscopic field. The bluish and reddish coloration could correspond to microbiological stains used to highlight cell walls and reproductive structures. Although the 100x magnification limits the detailed observation of complete conidiophores, the morphology observed is consistent with *Aspergillus spp*. The photomicrograph reveals the interaction at a microscopic level. Filamentous structures (hyphae) are observed interwoven on the polymer matrix. The presence of these

structures confirms that the fungus is not only present on the surface, but is interacting intimately with the material's surface.

The results suggest that *Aspergillus salvadorensis* has the ability to colonize polymeric surfaces and generate fungal biomass on degraded materials. The growth observed after 21 days could be related to biodeterioration processes, biofilm formation and possible enzymatic degradation of the polymer, phenomena frequently associated with the production of oxidative and hydrolytic enzymes in filamentous fungi. These findings support the biotechnological potential of the species in biodegradation studies and biological recycling of polymers.

Discussion

The genomic analysis carried out in *Aspergillus salvadorensis* allowed the identification of a set of genes associated with hydrolytic enzymes potentially involved in the degradation of polymers, which suggests that this species has a specialized metabolic machinery for the transformation of macromolecular compounds present in its environment. The detection of the open reading frames ORF18 and ORF2, which encode domains belonging to the superfamily α/β hydrolases, constitutes important evidence of the degradative potential of the organism. The enzymes belonging to this superfamily are characterized by a highly conserved structural architecture that includes a folded core α/β and an active site composed of the catalytic triad Serine–Histidine–Aspartic Acid, responsible for the hydrolysis of ester bonds in a wide variety of organic substrates (Bornscheuer, 2002).

The GTSAG pentapeptide motif identified in the analyzed sequences is consistent with catalytic regions present in cutinase, lipase or esterase enzymes, which have been widely described in filamentous fungi of the genus *Aspergillus*. These enzymes play a critical role in the degradation of natural polymers such as cutin, suberin, and other plant-based structural compounds, allowing fungi to access complex carbon sources present in the environment (Bennett, 2010). The presence of these functional domains in *A. salvadorensis* suggests that this species could possess a similar or even expanded metabolic capacity for the degradation of both natural and synthetic polymeric materials.

In the context of the degradation of synthetic polymers, several studies have shown that enzymes belonging to the family of cutinases and microbial esterases can hydrolyze ester bonds present in polyesters such as polyethylene terephthalate (PET) and other petroleum-based plastics (Tokiwa et al., 2009). The identification of α/β hydrolase domains with exposed catalytic centers in the genome of *A. salvadorensis* suggests that these enzymes could interact effectively with solid polymeric surfaces, facilitating biodegradation processes. This aspect is particularly relevant from an environmental perspective, given that plastic waste represents one of the most persistent pollutants in natural ecosystems.

The experimental results obtained in this study showed that the degradation of polyester is significantly increased when the material is subjected to oxidative pretreatment with hydrogen peroxide (H_2O_2). This chemical treatment likely induces structural modifications in the polymer matrix, such as carbonyl group formation or partial bond breaking, which increases the material's susceptibility to subsequent enzymatic attack. Previous research has indicated that oxidation or photooxidation processes can increase the biodegradability of plastics by generating reactive sites that facilitate microbial colonization (Shah et al., 2008).

The formation of hydrolysis halos observed in the experimental plates is a classic indicator of extracellular enzymatic activity in degrading fungi. This phenomenon reflects the active secretion of hydrolases into the surrounding environment, where they interact with the polymeric substrate and generate degradation products of lower molecular weight. In filamentous fungi, the secretion of extracellular enzymes represents a fundamental metabolic strategy for the acquisition of nutrients from insoluble substrates or those of high structural complexity (Lynd et al., 2002). In this sense, the ability of *A. salvadorensis* to produce hydrolysis halos suggests an efficient system of enzymatic secretion adapted to the degradation of complex materials.

The mass loss of more than 30% observed in samples subjected to oxidative pretreatment and fungal exposure represents a remarkable result compared to other plastic biodegradation studies, in which degradation rates are usually considerably lower or require longer incubation periods. This result suggests that the combination of physicochemical treatments and biological activity may constitute an effective strategy to accelerate the transformation of polymeric waste in the environment.

From an ecological perspective, the presence of polymer-degrading enzymes in the genome of *A. salvadorensis* could reflect an evolutionary adaptation to the tropical ecosystems where this species thrives. Tropical ecosystems, characterized by high temperatures, high humidity, and abundant plant biomass production, are home to a great diversity of microorganisms capable of breaking down complex organic compounds (Peay et al., 2016). In these environments, filamentous fungi play a critical role in nutrient recycling and mineralization of plant structural compounds.

The potential ability of *A. salvadorensis* to degrade synthetic polymers could be derived from its prior adaptation to the degradation of natural polymers present in the environment, such as cutin, lignin or cellulose. This phenomenon, known as functional cooptation, suggests that enzymes originally intended to degrade natural compounds may acquire the ability to transform structurally similar synthetic materials (Shah et al., 2008).

Finally, the results of this study position *A. salvadorensis* as an organism of interest for the development of bioremediation strategies aimed at the degradation of plastic waste. The identification of hydrolytic enzymes with degradative potential opens up new opportunities for their application in biotechnological processes, including the production of industrial biocatalysts, the treatment of plastic waste and the conversion of polymers into compounds with a lower environmental impact. Future research should focus on the detailed biochemical characterization of the identified enzymes, the determination of their three-dimensional structure, and the evaluation of their catalytic efficiency against different types of synthetic polymers.

Conclusion

The present research comprehensively demonstrates that the degradation of polyesters in *Aspergillus salvadorensis* is a real, efficient and sustained biological process both at the genomic and experimental levels. Bioinformatic analysis of the sequence allowed to clearly identify two key open reading frames, ORF18 and ORF2, which together encode characteristic catalytic domains of the α/β hydrolase superfamily. The presence of the conserved GTSAG motif confirms the existence of an essential nucleophilic serine, typical of enzymes such as cutinases and esterases, specialized in the breaking of ester bonds in polymers.

From an experimental point of view, the results show that the activity of these enzymes depends directly on the accessibility of the substrate. Macroscopic observations showed the formation of well-defined hydrolysis halos at the interface between the fungus and the plastic, confirming the active secretion of enzymes capable of destructuring polyester. This phenomenon was significantly intensified when the material was subjected to a pretreatment with 10% hydrogen peroxide, which modified the surface of the polymer, making it rougher and more porous. This alteration facilitated both the adhesion of the mycelium and the exposure of the ester bonds to enzymatic attack.

Genomic and experimental data converge on the same conclusion: *Aspergillus salvadorensis* has a functional, robust and highly specialized enzymatic machinery for the degradation of polyesters. This ability is not accidental, but the result of a precise genetic organization, where mechanisms such as splicing allow the assembly of the domains necessary to form a complete, stable hydrolase secreted into the extracellular medium.

It is also established that pretreatment with 10% hydrogen peroxide is a key element within the process, as it acts as a structural facilitator that "unlocks" the polymer matrix. By generating microcracks and increasing the reactive surface, it allows catalytic serine to more efficiently access chemical bonds. The formation of fungal biofilms in these areas further enhances the process, by concentrating the enzymatic activity directly on the substrate.

In summary, this research confirms that the degradation of polyesters by *Aspergillus salvadorensis* is the result of a coordinated strategy between chemical and biological factors. The synergy between oxidative pretreatment and enzymatic action significantly reduces the energy barriers of the process, allowing accelerated degradation. These findings position this strain as a promising biotechnological tool, with potential application in the development of sustainable solutions for the bioremediation of plastic waste in the current environmental context.

Gratitude and Recognition

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Conflicts of Interest

The author declares that he has no conflict of interest.

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