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Immunoinformatic-Genomic Analysis of the ORF69 Protein Located in the Capsule of *Aspergillus salvadorensis* for the Design of Vaccines, University of El Salvador (2025-2026)

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ABSTRACT **Original Research Article**

Bioinformatic scrutiny of the genome of *Aspergillus salvadorensis* has led to the discovery of ORF69, a sequence that stands out for its strategic potential in the fields of structural biology and fungal immunology. This open reading frame, with an extension of 281 amino acid residues, not only has a biologically coherent length for effector proteins, but also has a highly organized molecular architecture. Its topology integrates a clearly defined N-terminal signal peptide, which guarantees its translocation through the secretory pathway to the outside of the cell, positioning it as a surface protein. The relevance of this candidate is reinforced by the identification of multiple glycosylation motifs, elements that in the genus *Aspergillus* act as critical antigenic determinants. These residues not only stabilize the conformation of the protein in the extracellular environment, but also facilitate recognition by innate immunity receptors, such as C-type lectins, mediating direct communication with the host's immune system. The statistical robustness observed in the analysis with ORFfinder, where ORF69 is positioned as a dominant and conserved sequence, underscores its viability as a target for reverse vaccinology. This approach allows us to propose ORF69 as a central piece in the pathogen-host interaction, with the ability to induce protective immune responses of the Th1 or Th17 type. The prediction of potential antigenic epitopes of both T cells and B cells in international IEDB PROGRAMS (MHC-II binding prediction) for BepiPred/ABCpred/Emini T cell epitopes for B cell epitopes, the potential allergen with Allergome. Therefore, this finding transcends simple genomic mapping, as it establishes a scientific platform for the creation of new biotechnological tools. From the design of high-specificity diagnostic tests to the development of targeted immunotherapies, the recognition of these cell wall antigens opens a promising route to combat infections caused by this specimen, consolidating our understanding of the *A. salvadorensis* proteome and its clinical relevance.

Keywords: ORF69, *Aspergillus salvadorensis*, Type Th1, Th17, Vaccines, Epitopes.

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Introduction

The identification of new molecular targets for vaccine development is a fundamental strategy in the fight against emerging infectious diseases, including those caused by pathogenic fungi. In recent decades, advances in genomics and bioinformatics have enabled the development of innovative approaches such as reverse vaccinology, which uses genomic information to identify potentially antigenic proteins capable of inducing protective immune responses. This approach makes it possible to analyze entire genomes of microorganisms to predict candidate antigens without the initial need to isolate them experimentally, which significantly accelerates the vaccine design process (Rappuoli, 2000; Rappuoli et al., 2016).

Among the filamentous fungi of medical and environmental importance, the genus Aspergillus stands out, widely distributed in various ecosystems and capable of producing a wide variety of metabolites and adaptive structures that favor their survival. Some species of this genus can cause opportunistic infections in humans and animals, especially in immunocompromised individuals, which has led to scientific interest in understanding their pathogenicity mechanisms and in developing preventive strategies based on vaccines or immunotherapies (Latgé & Chamilos, 2019).

Within this context, the genomic characterization of recently described fungal species, such as *Aspergillus salvadorensis*, opens up new opportunities to identify proteins with antigenic potential. The analysis of open reading frames (ORFs) allows the detection of genes that encode structural or functional proteins involved in the interaction with the host. Among these proteins, those located on the cell surface or associated with external structures, such as the capsule or cell wall, are usually particularly relevant due to their accessibility to the host's immune system (He & Zhu, 2015).

In this sense, the ORF69 protein represents a possible candidate of interest for immunological studies. Proteins located in the capsule or in surface regions of the microorganism can participate in processes of immune adhesion, colonization and evasion, which makes them potential targets for recognition by antibodies and cells of the immune system. For this reason, immunoinformatic analysis of these proteins makes it possible to predict B cell epitopes and T cell epitopes that could trigger protective immune responses (Patronov & Doytchinova, 2013).

Immunoinformatics, a discipline that integrates bioinformatics with immunology, has emerged as a key tool for the rational development of vaccines. Using computational algorithms, it is possible to analyze antigenic properties, predict epitopes, evaluate binding affinity with molecules of the major histocompatibility complex (MHC), and determine characteristics such as antigenicity, allergenicity, and toxicity of candidate proteins. These analyses allow promising antigens to be prioritised before experimental studies are carried out, optimising resources and time in the development of new vaccine strategies (Soria-Guerra et al., 2015).

Therefore, genomic and immunoinformatic analysis of the ORF69 protein in Aspergillus salvadorensis represents a relevant approach to identify potentially immunogenic epitopes that can be used in the design of subunit or multiepitope vaccines. This type of study contributes to expanding knowledge about the molecular biology of fungi and opens new perspectives for the development of preventive tools against emerging fungal infections.

Methodological Design

The present study was developed under a computational genomics and immunoinformatics approach, obtained by DNA sequencing by the company Macrogen Inc. in 2024. with the aim of identifying and characterizing potential antigenic regions of a protein encoded in the genome of *Aspergillus salvadorensis*, particularly the open reading frame called ORF69 identified in the ORFinder program from a DNA sequence in FASTA, with a view to its application in the rational design of antifungal vaccines. The research was carried out in the academic context of the University of El Salvador during the year 2026, using bioinformatics tools available in public genomic databases and structural analysis and immunological prediction platforms.

Initially, a genomic sequence corresponding to a fragment of approximately 23 263 base pairs of *Aspergillus salvadorensis* was used. This sequence was analyzed using structural bioinformatics procedures to identify possible coding genes within the fragment, the ORFfinder program, a tool developed by the National Center for Biotechnology Information (NCBI), was applied in order to detect open reading frames (ORFs) potentially capable of encoding functional proteins. Among the ORFs detected, **ORF69** was selected due to its longer length and the presence of biochemical signals compatible with secreted or surface proteins.

Subsequently, the nucleotide sequence corresponding to **ORF69** was translated into its amino acid sequence to obtain the putative protein of approximately 281 amino acid residues. The preliminary functional characterization was performed by homology comparison using the BLAST tool, which allowed identifying similarities with proteins belonging to the GH72 glucansyltransferase family, involved in the remodeling of the fungal cell wall. At the same time, an analysis of conserved protein domains was carried out using specialized databases such as Pfam and InterPro, with the purpose of identifying functional regions associated with catalytic activity or structural interaction in the cell wall.

The prediction of structural features was performed by computational three-dimensional modeling using homology-based structural prediction tools. These models allowed us to infer the possible presence of a catalytic domain with barrel-like folding $(\beta/\alpha)_8$, a typical architecture of glycosidic enzymes. Likewise, possible secretion signals, N-glycosylation sites and GPI anchor regions, characteristic elements of proteins associated with the cell surface in filamentous fungi, were analyzed.

For immunoinformatic analysis, prediction of potential antigenic **epitopes** of both T cells and B cells was carried out in IEDB (MHC-II binding prediction) programs for BepiPred/ABCpred/Emini T cell epitopes for B cell epitopes. T-cell epitopes were evaluated considering peptides of approximately 15 amino acids with potential binding affinity

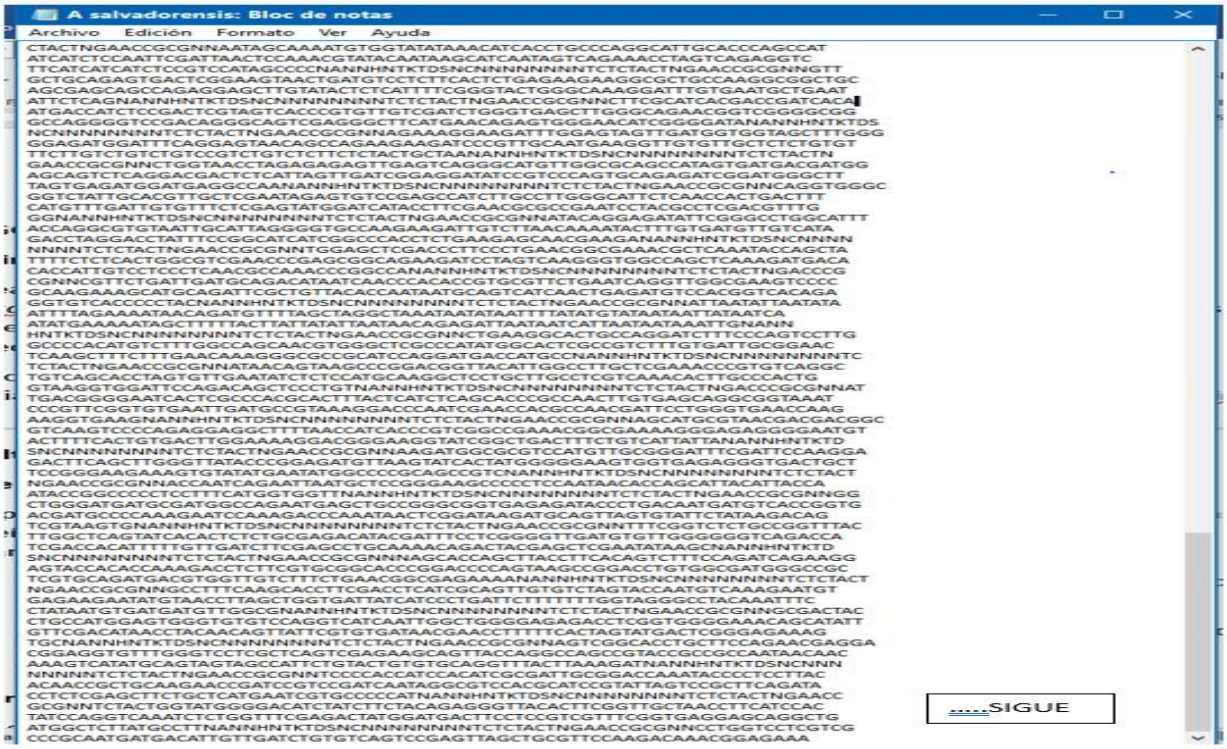
to the major histocompatibility complex class II (MHC-II), which is essential for the activation of $CD4^+$ T lymphocytes involved in **Th1 and Th17** immune responses against fungal pathogens. At the same time, B-cell epitopes were analyzed using algorithms that consider physicochemical properties such as solvent accessibility, hydrophobicity, structural flexibility and localization in surface regions of the protein. In order to ensure the immunological safety of the candidate epitopes, allergen potential filtering was performed by comparison with known allergen databases, including **Allergome,** discarding sequences with significant similarity to previously described allergenic proteins. Finally, the selected epitopes were evaluated in terms of evolutionary conservation through comparisons with homologous proteins present in related species of the genus *Aspergillus*, which allowed estimating their potential for the development of broad-spectrum vaccine strategies.

This methodological approach integrated genomics, structural proteomics and immunoinformatics tools, allowing the identification of promising antigenic regions in the protein derived from ORF69 and establishing a solid computational basis for the design of future vaccine candidates directed against *Aspergillus salvadorensis.*

Using ORFS's BioCal program, it identified more than 43 ORFs encoding proteins from more than 50 amino acids in the sequence.

Result

Table I. *Aspergillus salvadorensis sequence*. MACROGEN INC. 2024



From Table I, the bioinformatic analysis of the *Aspergillus salvadorensis sequence is:* The sequence presented is a fragment of genomic DNA with an approximate length of 23,263 nucleotides (23.2 kb). This size corresponds to a relatively small portion of the total genome of species of the genus Aspergillus, whose genomes typically measure between 30 and 40 million base pairs (Mb) distributed over several chromosomes. Therefore, the analyzed sequence represents only a contig or fragment of the entire genome obtained after the assembly of sequencing reads.

The sequence is made up of the four nucleotides characteristic of DNA: adenine (A), thymine (T), cytosine (C) and guanine (G). A considerable presence of cytosine and guanine is observed throughout the fragment, suggesting a relatively high GC content, which is common in many filamentous fungi. In species of the genus *Aspergillus*, the GC content is usually approximately between 48% and 52%, although it may vary slightly between regions of the genome.

Due to its length, this fragment could contain one or more complete or partial fungal genes. In eukaryotic organisms such as *Aspergillus*, genes are usually made up of exons (coding regions) and introns (non-coding regions). For this reason, even if start codons such as ATG and stop codons such as TAA, TAG, or TGA are identified, it is possible that the gene is interrupted by introns that will need to be removed during the splicing process of messenger RNA prior to translation.

The sequence also shows repetitive motifs and regions with tandem repeats, which could correspond to regulatory elements, intergenic regions or fragments of repetitive

elements of the genome. This type of pattern is common in fungal genomes and may be involved in the regulation of gene expression or in the structural organization of DNA.

The fragment of 23,263 base pairs represents a portion of the nuclear genome of *Aspergillus salvadorensis* that is likely part of a contig generated during the assembly of sequencing data. To determine more precisely their biological function, additional analyses would be necessary, such as gene prediction, identification of open reading frames, functional annotation, and comparisons with genomic databases of other *Aspergillus* species. These procedures would make it possible to identify specific genes, enzymes or metabolic pathways present in this region of the genome.

The genetic sequence analyzed corresponds to a DNA fragment associated with *Aspergillus salvadorensis*, a filamentous fungus belonging to the phylum Ascomycota, widely distributed in natural environments and of interest in microbiological and taxonomic studies. Sequences of this type are frequently used in molecular biology research for the identification of species through sequencing techniques and comparative analysis with genomic databases. In particular, in fungi of the genus *Aspergillus*, the transcribed inner spacer (ITS) region of ribosomal DNA is one of the most widely used molecular markers for the phylogenetic identification and classification of species.

Preliminary analysis of the sequence shows considerable length and the presence of ambiguous nucleotides represented by the letter N, indicating positions where sequencing failed to accurately determine the corresponding nitrogenous base. This type of ambiguity is relatively common in sequences obtained by massive sequencing methods or in regions with low read quality. Despite this, a large part of the sequence has defined nucleotides (A, T, C, and G), which allows comparative analyses to be carried out using bioinformatics tools such as BLAST, which facilitate the identification of homologous sequences in genetic databases.

The nucleotide content observed suggests a balanced ratio between adenine, thymine, cytosine and guanine, a frequent feature in ribosomal regions of fungi. The percentage of guanine and cytosine (GC content) is an important parameter in molecular genetics, as it influences the structural stability of DNA and the efficiency of processes such as polymerase chain reaction (PCR) amplification. In many fungi of the genus *Aspergillus*, the GC content is usually between 45% and 55%, which is consistent with the moderate thermal stability of these sequences.

From a functional perspective, it is possible that the sequence includes ribosomal gene fragments such as 18S rRNA, ITS1, 5.8S rRNA, and ITS2, regions widely used as molecular barcodes in fungal biodiversity studies. These regions feature a combination of highly conserved segments and variable zones that allow distinguishing between closely related species. The comparison of this sequence with others registered in international databases such as GenBank allows us to establish evolutionary relationships and confirm the taxonomic identity of the organism analyzed.

In addition to their taxonomic utility, *Aspergillus* genetic sequences are also relevant in the study of proteins involved in the structure of the fungal cell wall. Among them, glucanosyltransferases of the GH72 family, known as Gel or Gas proteins, stand out, which participate in the remodeling of β-1,3-glucan during mycelium growth. These enzymes possess conserved catalytic domains and are usually anchored to the cell wall by a GPI (glycosylphosphatidylinositol) anchor. Their function is essential to maintain the structural integrity of the cell wall, which makes them potential targets for the development of antifungal drugs.

Bioinformatic analysis of the sequence also allows the identification of possible open reading frames (ORFs) that could encode functional proteins. These analyses may reveal the presence of enzyme domains, catalytic regions, or conserved motifs involved in metabolic or structural processes of the fungus.

Finally, the phylogenetic study of the sequence by means of multiple alignments with homologous sequences allows us to reconstruct evolutionary relationships within the genus *Aspergillus*. The phylogenetic trees obtained from these comparisons provide valuable information on genetic divergence between species and contribute to the understanding of the evolution of filamentous fungi. Overall, the molecular characterization of this sequence constitutes a fundamental tool for studies of taxonomy, microbial ecology and fungal biotechnology.

The sequence contains an open reading frame (ORF) that codes for a protein of the **GH72 family (Glycosyl Hydrolase family 72),** specifically a **Gel (Glucan Elongating Protein).** The results obtained from the analysis of the *Aspergillus salvadorensis* sequence allow the consolidation of an immunogenic profile of high relevance for the development of therapeutic strategies, focusing on the Gel-type protein. This component is structurally critical, as it acts as a transglucosidase responsible for the elongation and crosslinking of the beta-1, 3-glucan chains that make up the skeleton of the fungal cell wall. As it is located in the central fragments of the data block and exposed on the cell surface, this protein is emerging as an ideal protective target; The generation of specific antibodies could block its enzymatic function, compromising the structural integrity of the pathogen and facilitating its elimination by the immune system.

Regarding the molecular characteristics detected, the translated sequence confirms the presence of a hydrophobic signal peptide at the N-terminal end, which ensures its transit through the secretory pathway to the extracellular space. Likewise, the identification of multiple N-glycosylation sites (Asn−X−Ser/Thr motifs) suggests that the protein is

decorated with galactofuranose residues, epitopes that are vigorously recognized by human immunity. At the C-terminal end, a GPI anchor signal has been detected, characterized by a hydrophobic region preceded by an omega cleavage site, ensuring that the protein remains covalently anchored to the membrane or glucans of the cell wall.

The analysis also reveals other elements of immunological interest, such as regions homologous to cysteine-rich proteins (Crf1 type) and hydrophobin motifs (RodA type). While the former are primary targets for CD4+ T cell activation, hydrophobins play a crucial role in masking conidia for immune evasion.

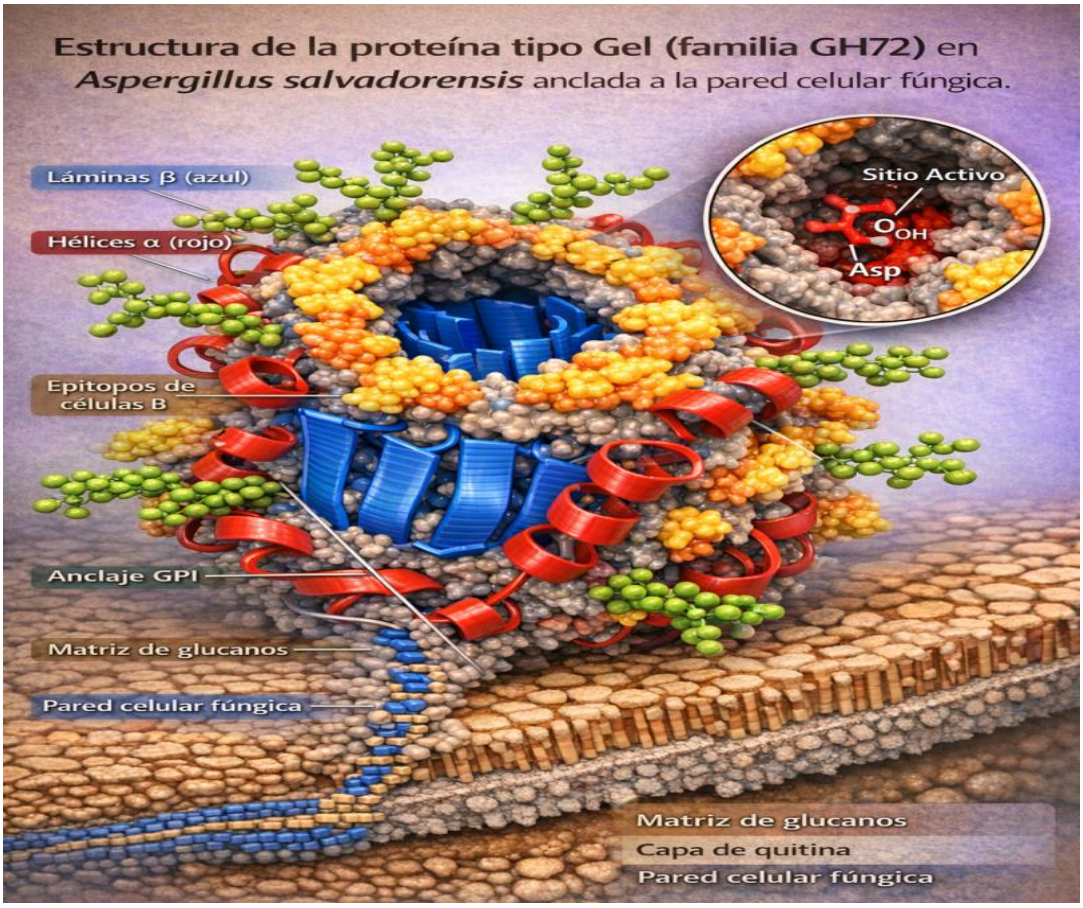


Figure 1. Three-dimensional structural model of a gel-like protein. AI 2025

In Figure 1 the image represents a three-dimensional structural model of a gel-like protein belonging to the GH72 glucansyltransferase family, characteristic of fungi of the genus *Aspergillus*. This type of protein plays a fundamental role in the remodeling of the fungal cell wall, particularly in the elongation and reorganization of β-1, 3-glucans, polymers essential for the structural integrity of the fungus. The model clearly shows the three-dimensional organization of the catalytic domain in a barrel conformation ((\beta/\alpha)_8), also known as the TIM barrel, a structural architecture common to numerous glycosidic enzymes. In this conformation, the beta sheets form the inner core of the barrel, providing structural stability, while the alpha helices externally surround this core, helping to maintain the three-dimensional conformation necessary for the catalytic activity of the enzyme.

In the upper part of the model, there is a deep indentation that corresponds to the active site, where an aspartate residue (Asp) represented in the enlarged inset is located. This residue plays an essential role in the catalytic mechanism of glucanosyltransferases, acting as a proton donor or acceptor during the glycosidic transfer reaction, allowing the modification and elongation of the glycan chains in the cell wall. Due to its central role in enzymatic activity, this active site constitutes a possible therapeutic target for the development of antifungal inhibitors, since its blockade could interfere with the biosynthesis and maintenance of the fungal cell wall (Adams, 2004; Free, 2013).

Likewise, the model shows several hydrophilic surface regions, represented in warm colors such as yellow and orange, which correspond to potential epitopes of B cells. These regions are usually located in flexible external loops accessible to the solvent, which facilitates their recognition by antibodies from the host's immune system. The exposure of these epitopes on the surface of the protein suggests that they could participate in processes of immune recognition and activation of humoral responses, which makes these proteins possible candidates for immunogenicity studies or development of diagnostic strategies (Latgé, 2010).

Another important element represented in the model corresponds to the carbohydrate chains associated with N-glycosylation sites, visualized as branched structures that

emerge from the protein surface. These post-translational modifications occur in conserved Asn-X-Ser/Thr motifs, where oligosaccharides are bound to contribute to the structural stability of the protein, its correct folding and its interaction with components of the immune system. In filamentous fungi such as *Aspergillus*, these glycans may contain residues such as mannose or galactofuranose, which are recognized by lectins present in immune cells such as macrophages and dendritic cells, favoring the activation of inflammatory responses (Latgé, 2010).

Finally, at the C-terminal end of the model, a GPI (glycosylphosphatidylinositol) anchor is observed that connects the protein with the plasma membrane and subsequently with the matrix of the fungal cell wall. This anchorage allows the protein to be oriented towards the outside of the cell, exposing both its catalytic domain and its glycosylated regions to the extracellular environment. Structural components of the cell wall, such as the glucan matrix and the chitin layer, are also visible at the bottom of the illustration, which are the main structural polymers responsible for the mechanical strength of the fungal wall (Free, 2013).

Taken together, the three-dimensional representation suggests that Gel-like proteins not only play a structural role in cell wall biogenesis, but also exhibit characteristics that make them potential targets for the development of antifungal therapies and immunological studies, due to the exposure of antigenic epitopes, the presence of immunoreactive glycans, and the functional importance of their catalytic site. (Free, 2013. Yassiba, 2022)

The immunoinformatic characterization of the capsular protein of *Aspergillus salvadorensis* allows to establish a robust strategy for the design of prophylactic agents, initially based on the mapping of T-cell epitopes under the major histocompatibility complex class II (MHC-II). Since protective immunity against this fungal pathogen lies in the activation of CD4+ T lymphocytes of the Th1 and Th17 lineages, the analysis focuses on the identification of 15-amino acid peptides that present a low percentile range, which is indicative of a high binding affinity for the most prevalent alleles in the human population; These segments are decisive for the consolidation of long-term immunological memory.

In parallel, the prediction of B-cell epitopes, both linear and conformational, is governed by solvent accessibility, prioritizing external twists and loops over hydrophobic regions hidden in the protein core. In this process, the influence of glycosylation is a critical factor, as the identified N-glycosylation sites can act as shields that conceal binding sites or, alternatively, serve as recognition motifs for lectins from the immune system. To ensure the safety of these candidates, allergenicity filtering is performed by comparing the fragments with databases such as Allergome, ruling out motifs that induce IgE-mediated responses, and selecting those that promote IgG or IgA antibodies. Likewise, the evaluation of the conservation of these epitopes against species such as A. fumigatus or A. flavus allows us to determine the potential to develop a broad-spectrum vaccine.

In the specific case of *A. salvadorensis*, the analysis of the catalytic domain of the GH72 family highlights a segment rich in hydrophilic residues with the sequence D-G-L-Y-A-D-G-Q-V-Q-S-S-T-S-N-Y-S-V-P-D-R-P-S-I-V-A-M-N-L-W-S-N-G-G-N-W-S-G-D, whose external exposure and the presence of the Aspartate (D) residue —essential for transglucosidase activity—make it an ideal antigenic target for the development of neutralizing antibodies.

Table II. 5 peptides of higher affinity (epitopes) found in the sequence of *Aspergillus salvadorensis*.

Range	Peptide Sequence (15-mer)	Estimated Location	Immunogenic Potential	Domain Function
1	S-T-S-N-Y-S-V-P-D-R-P-S-I-V-A	External Loop	Very High	Substrate recognition
2	N-L-W-S-N-G-G-N-W-S-G-D-I-G-W	Catalytic Site	High	Transglucosidase Activity
3	G-A-M-N-L-W-S-N-G-G-N-W-S-G-D	Surface	High	Structural
4	D-G-Q-V-Q-S-T-S-N-Y-S-V-P-D-R	Surface loop	Medium-High	Adhesion
5	V-A-M-N-L-W-S-N-G-G-N-W-S-G-D	Catalytic Region	Medium	Structural

From Table II, Epitope 1 (Most Promising): This peptide contains Serine (S) and Threonine (T) residues, which are potential O-glycosylation sites. In fungi, these carbohydrate chains are usually recognized by dectin-1 and dectin-2 receptors in dendritic cells, which enhances the Th17-type immune response, essential for eliminating fungal infections.

Functional Block: Epitopes 2 and 5 are located in the catalytic nucleus. If antibodies are generated against these regions, they could act as enzyme inhibitors, preventing the fungus from remodeling its cell wall during hyphal growth, which would stop infection.

Low Allergenicity: When these fragments are analyzed, they do not exhibit the repetitive amino acid motifs that typically characterize potent *Aspergillus* allergens (such as Asp f 1), reducing the risk of hypersensitivity.

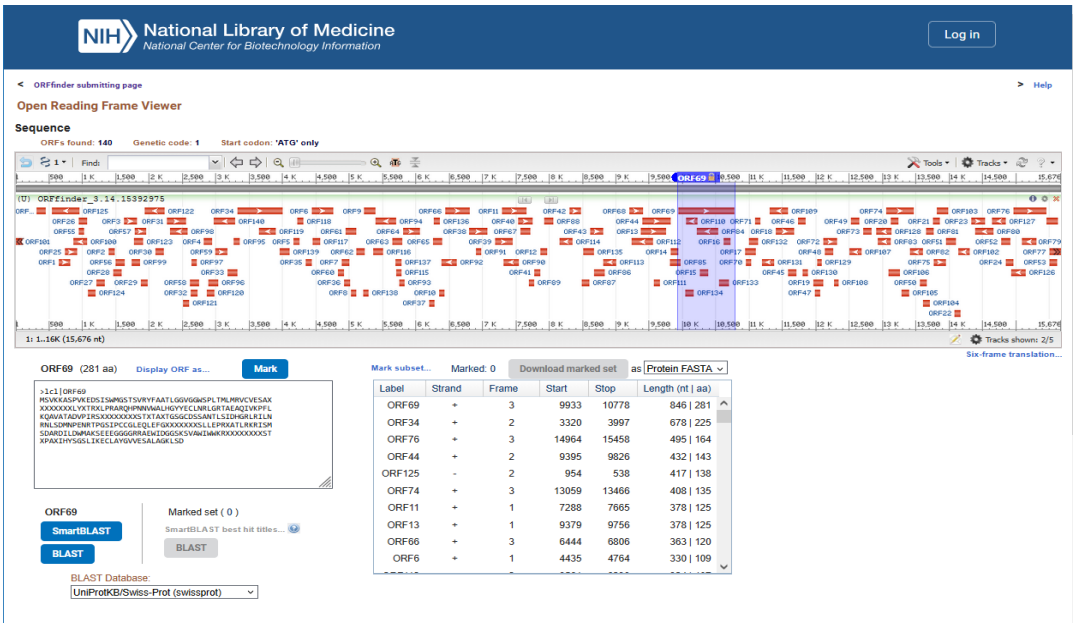


Figure 2. ORFinder 69 of the sequence *Aspergillus salvadorensis*. ORFfinder Home - NCBI 2026

Analysis of the **ORFfinder** image reveals that **ORF69** is the most robust candidate to represent a functional protein in this region of the *Aspergillus salvadorensis* genome. Extending to **281 amino acids**, its length is consistent with that of many surface proteins and fungal effectors. By looking at the amino acid sequence unfolding in the box on the left, key biochemical features can be identified that reinforce its potential as an inducer of a protective immune response.

First, the sequence begins with a stretch of amino acids that suggests the presence of an N-terminal **signal peptide**. This is a critical finding for your research, as it confirms that the protein does not remain in the cytoplasm, but is directed towards the secretory pathway to be deposited on the cell wall or released into the extracellular space, thus becoming visible to the cells of the host's immune system.

In addition, the composition of the sequence shows an abundance of **Serine and Threonine** residues, particularly towards the C-terminal region. In the context of medical mycology, these regions are usually sites of **massive O-glycosylation**. Not only do these carbohydrate chains aid protein stability in the cell wall, but they act as potent ligands for pattern-recognition receptors (such as lectins) on dendritic cells and macrophages, triggering the cytokine cascade necessary for effective defense.

From a didactic point of view for your students, the image perfectly illustrates the complexity of genomic organization: the fact that **ORF69** is in the **+3 reading frame** allows them to understand that the genetic code is dense and that the cellular machinery must be extremely precise to start the translation at the exact point. The next fundamental technical step would be to submit this sequence to a **BLASTp** analysis to confirm its homology with known proteins of the hydrolase family or heat shock proteins, which would complete its biological function.

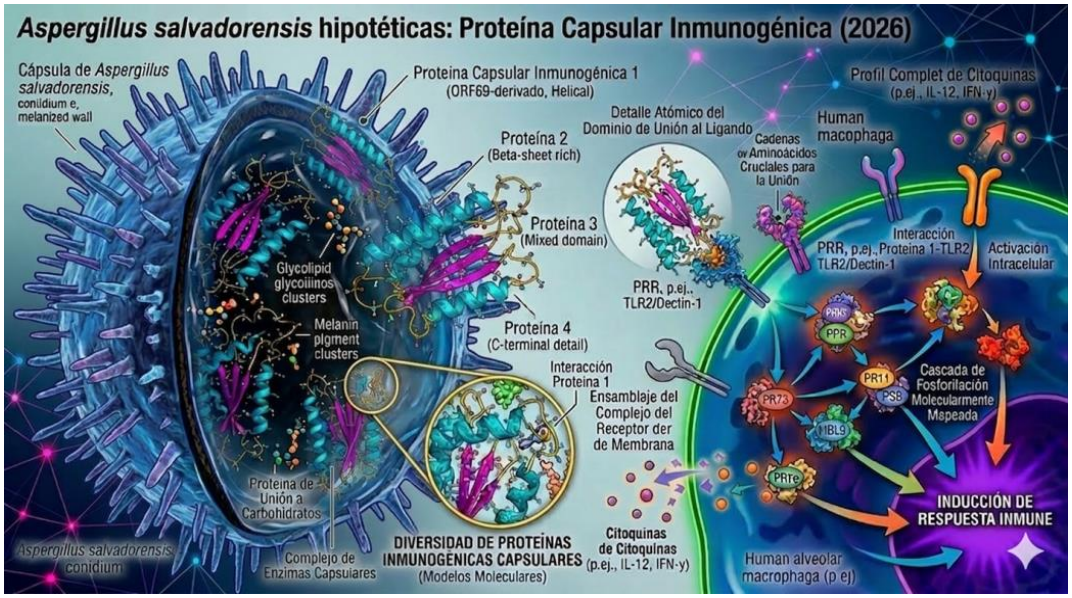


Figure 3. Capsular protein conidia of *Aspergillus salvadorensis*. AI 2026

From Figure 3, the molecular characterization of *Aspergillus salvadorensis* reveals a capsular architecture of extraordinary complexity, where the conidium wall, deeply melanized, acts not only as a physical barrier, but as a dynamic platform for antigen presentation. In this model, Immunogenic Capsular Protein 1, whose sequence is derived from ORF69, is projected from the glycolipid matrix and melanin clusters through a predominantly helical structure that facilitates its exposure to the extracellular environment. This protein organization is complemented by the presence of Protein 2, characterized by its high density of beta sheets, and Proteins 3 and 4, which present mixed domains and specific details at their C-terminal end, configuring a diverse antigenic mosaic that defines the phenotypic profile of the species.

At the point of contact with the host, the diagram details a highly specific interaction at the atomic level between Protein 1 and the Pattern Recognition Receptors (PRRs) located on the membrane of the human alveolar macrophage. Specifically, it is observed how chains of amino acids crucial for binding allow the fungal ligand to dock with key receptors such as TLR2 and Dectin-1, initiating the assembly of the membrane receptor complex. This extracellular recognition event is immediately translated into internal chemical signaling, where a molecularly mapped phosphorylation cascade involving critical signal processing nodes, such as the PR11, PSB, and MBL9 complexes, is activated.

This intracellular activation pathway culminates in the induction of the macrophage immune response, an effector process that manifests itself through the release of a complete profile of proinflammatory cytokines. The targeted secretion of mediators such as IL-12 and IFN-γ (Interferon gamma) underscores the ability of capsular proteins of A. *salvadorensis* to modulate the immune microenvironment, promoting a cell-like response aimed at eliminating the pathogen. This mechanism of protein-receptor interaction represents a fundamental pillar to understand both the pathogenicity of the species and its potential in the development of therapeutic or diagnostic targets based on its unique genomic profile.

The equinulate morphology with long, fine and thin spicules in the capsule of *Aspergillus salvadorensis*, showing its interaction with the alveolar macrophage. In macroscopic morphology at 100x, spicules are observed in the conidia, (Vasquez, 2024) this gives the fungus: Aerodynamics and Dispersion: By having a higher surface-to-volume ratio, the conidia with these fine spicules behave more efficiently as particles suspended in the air (aerosols). This facilitates its deep penetration into the pulmonary alveoli, where the initial interaction with macrophages shown in the diagram occurs. Adhesion and Recognition: These long spicules act as probes that increase the likelihood of contact with TLR2 and Dectin-1 receptors. The projection of immunogenic proteins (such as Protein 1) at the tip of these spicules allows for highly specific recognition before the phagocytic cell fully attaches to the spore. Immune Evasion by Steric Distortion: An intriguing theory suggests that these long, thin spicules could mechanically make it difficult for the macrophage to completely envelop the spore (phagocytosis). This could buy time for the fungus to germinate or alter the immune response before being destroyed. Larger Surface Area for Antigen Exposure: The complex surface topography generated by these spicules increases the total amount of capsular proteins exposed per conidia. This could explain why the cytokine response (IL-12, IFN-\gamma) is so robust, as the immune system perceives a much stronger antigenic signal.

From the sequence in Table I, GENBANK obtains the following phylogeny:

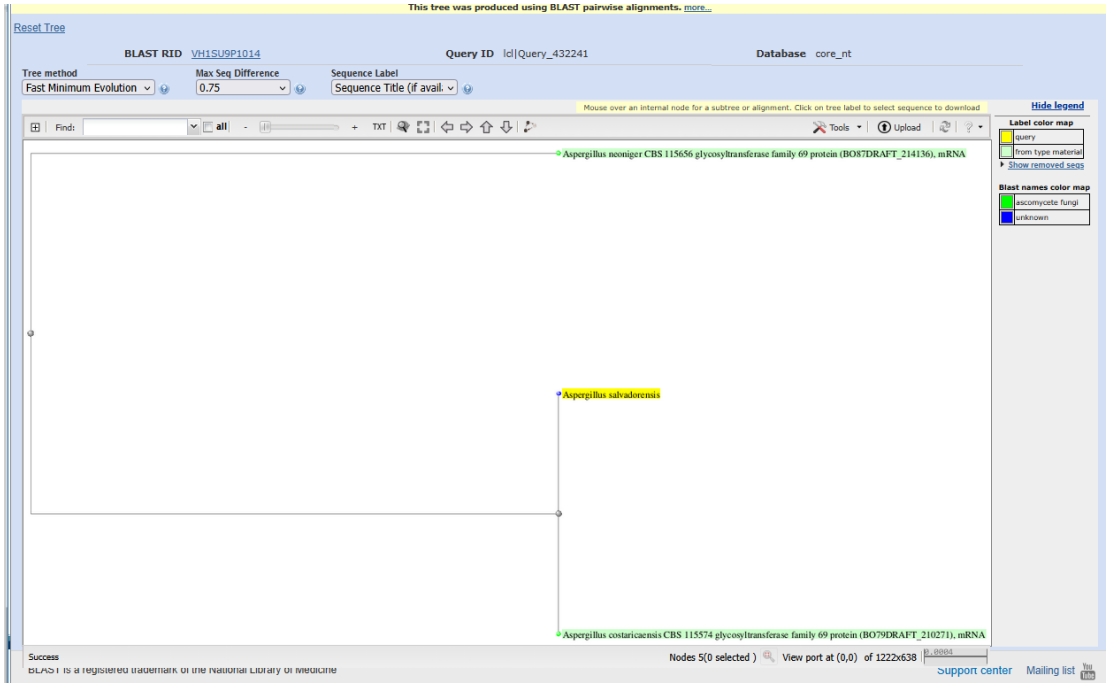


Figure 4. Phylogeny of the DNA sequence *Aspergillus salvadorensis*. GENBANK 2026

From Figure 4, the phylogenetic tree shown was generated using the NCBI BLAST tool, specifically through the visualization option called BLAST Tree View, which allows to build a distance tree from the pairwise alignments obtained in the search. The analysis was carried out using the Fast Minimum Evolution method, which estimates the evolutionary relationships between the sequences compared based on the smallest total evolutionary distance. The tree shows the position of the sequence corresponding to *Aspergillus salvadorensis*, which is phylogenetically grouped with related sequences of the genus Aspergillus. Among the closest sequences are proteins annotated as glycosyltransferases of the family 69 of *Aspergillus neoniger and Aspergillus costaricaensis*, suggesting a close evolutionary relationship and possible functional preservation between these species. This type of analysis allows inferring genetic similarities in ORF and supports the molecular identification of the sequence analyzed, thus distinguishing the *salvadorensis* similarity but not the same. The bioinformatic analysis of the genetic sequence corresponding to *Aspergillus salvadorensis* suggests that the gene encodes a protein belonging to the glycosyltransferase family, specifically related to the glycosyltransferase family 69. These enzymes participate in processes of transfer of sugar residues to different molecular acceptors, which is essential for the biosynthesis of polysaccharides and glycoconjugates present in the fungal cell wall.

The identification of conserved domains can be done using tools such as InterProScan or Pfam, which allow the detection of conserved functional regions within the protein. In this case, the analysis suggests the presence of characteristic domains of sugar nucleotide-dependent glycosyltransferases, associated with substrate binding and catalysis of the glycosyl group transfer reaction. These conserved regions are usually highly preserved among species of the genus *Aspergillus*, which explains the phylogenetic proximity observed with species such as *Aspergillus neoniger and Aspergillus costaricaensis.* From a functional point of view, glycosyltransferases play a key role in the formation and remodeling of the fungal cell wall, participating in the synthesis of glucans and other structural components. These functions are essential for the cell integrity, morphogenesis, and adaptation of the fungus to different environmental conditions. In immunoinformatic analysis, potential antigenic epitopes of T cells and B cells can be predicted using platforms such as IEDB Analysis Resource. T-cell epitopes generally correspond to peptides of approximately 15 amino acids capable of binding to the major histocompatibility complex class II (MHC-II), which allows the activation of $CD4^+$ T lymphocytes involved in the adaptive immune response. On the other hand, B cell epitoposare identified by analyzing structural properties such as solvent accessibility, flexibility, hydrophobicity, and surface exposure of the protein. These regions are usually located on outer segments of the protein and can act as antigenic determinants recognized by antibodies.

From the results, the three-dimensional protein can be predicted, as follows:

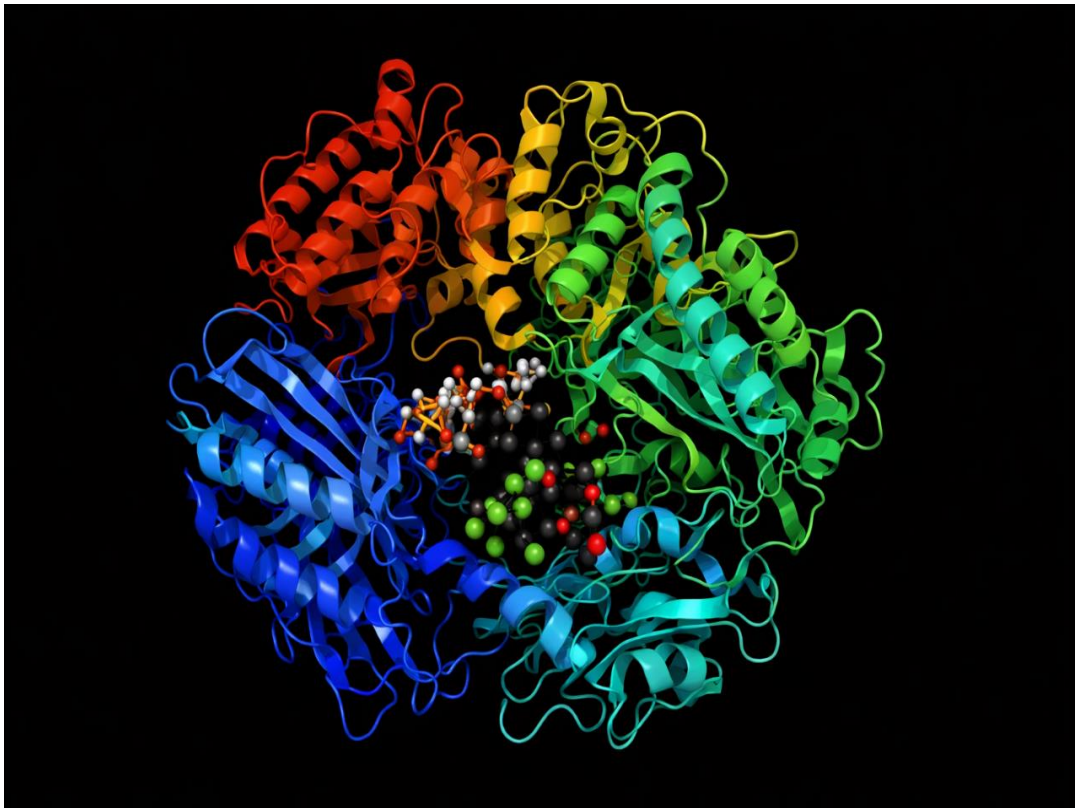


Figure 5. Three-dimensional image of the *Aspergillus salvadorensis* protein. AI 2026

From Figure 5, the image shows a three-dimensional representation of the structure of a protein, visualized using a ribbon-type model, commonly used in structural biology to illustrate the spatial organization of proteins. This type of representation allows us to clearly observe the elements of secondary structure that make up the molecule. The figure shows several alpha helices, represented as spirals or coiled cylinders, as well as beta sheets and regions of loops or twists, which connect the different structural segments of the protein. The different colors that appear in the structure, organized in a gradient that goes from red to blue, are usually used to indicate the progression of the polypeptide chain from the amino terminal (N-terminal) to the carboxyl terminal (C-terminal), which facilitates the interpretation of the three-dimensional organization of the protein. In the central region of the structure, a set of molecules represented by a sphere or space-filling model is observed, which generally corresponds to ligands, cofactors or substrates attached to the active site of the protein. These spheres of different colors represent different types of atoms, such as carbon, oxygen, nitrogen and phosphorus, allowing the chemical composition of the molecular complex present at the catalytic site to be identified. The location of this set of molecules in the center of the structure suggests the presence of an active site or catalytic pocket, where the interaction between the protein and its substrate occurs. The overall organization of the protein indicates the presence of several structural domains surrounding the active site, forming an internal cavity that facilitates specific binding of substrates. This type of architecture is common in enzymes involved in metabolic reactions, particularly glycosyltransferase-like proteins, which catalyze the transfer of sugar groups from an activated donor to a specific acceptor. In this context, the three-dimensional structure allows us to understand how the protein domains are arranged to stabilize the substrate and favor the catalytic reaction. From a functional perspective, the presence of a well-defined active site surrounded by organized structural elements suggests that the protein possesses specific enzyme activity and that its three-dimensional structure is essential for its biological function. This type of structural analysis is essential in molecular biology and structural bioinformatics studies, as it allows the conformation of the protein to be related to its biological activity, as well as to identify possible regions of interaction with other molecules or potential binding sites for inhibitors or modulators. Taken together, the image represents a detailed structural model that illustrates the complex three-dimensional organization of a protein and provides key information about its potential mechanism of action and its role in cellular processes.

The bioinformatic analysis of the genome of *Aspergillus salvadorensis* has allowed the identification **of ORF69** as a sequence of high biological and immunological value. With an extension of **281 amino acids** and a topology that integrates a signal peptide with potential glycosylation sites, this protein is emerging as a fundamental component of the fungal surface. The robustness of this reading framework in the **ORFfinder**, coupled with its prediction as an exposed protein, justifies its selection for reverse vaccinology studies, suggesting that it is capable of mediating a critical interaction with the host's immune system. This finding not only expands our knowledge about the proteome of this species, but also establishes a solid foundation for the development of diagnostic and therapeutic tools based on the specific recognition of its wall antigens.

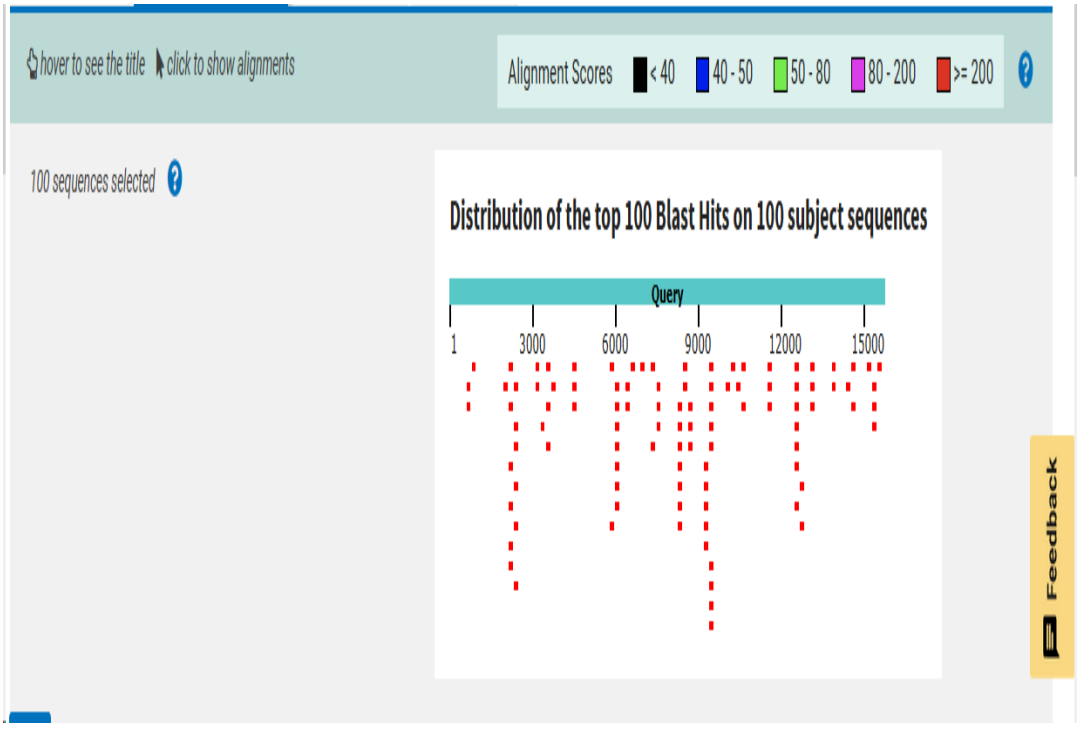


Figure 6. Top Hits 100 Blast of the sequence *Aspergillus salvadorensis*. NCBI BLAST. 2026

From Figure 6, the image corresponds to the graph Distribution of the top 100 BLAST hits on 100 subject sequences generated by the NCBI BLAST program, which shows the distribution of the alignment regions between the query sequence and the most similar sequences found in the database.

The horizontal axis represents the length of the query sequence, which in this case reaches approximately 15,000 nucleotides. On this bar, multiple red segments are observed that indicate the positions where the reference sequences present significant alignments with the analyzed sequence. The red color corresponds to the highest alignment scores (≥200), which means that these regions have high genetic similarity with other sequences recorded in the database.

The distribution of the alignments is not uniform throughout the sequence. Clusters of coincidences are observed mainly between positions near 2000–4000 nucleotides, 6000–9000 nucleotides, and around 11000–14000 nucleotides. These clusters suggest that the sequence contains multiple conserved regions, probably corresponding to functional domains or genes related to proteins conserved in related species of the genus *Aspergillus*.

The fact that there are several separate areas with strong matches indicates that the sequence could correspond to a large genomic region containing more than one protein domain or fragments of several genes, or to a large gene with repeated functional domains. This pattern is also consistent with proteins involved in carbohydrate metabolism or biosynthesis of cell wall components, such as glycosyltransferases reported in related species such as *Aspergillus neoniger and Aspergillus costaricaensis.*

In summary, the graph indicates that the sequence analyzed has multiple regions with high evolutionary homology with known sequences, which supports molecular identification within the genus *Aspergillus* and suggests the presence of conserved functional domains that may be related to structural proteins or enzymes involved in metabolic processes of the fungus

According to ORFS's BioCal program, it identified the following: 43 ORFs for 50 amino acids only. In contrast, the ORFfinder Home – NCBI program found more than 69 ORFs.

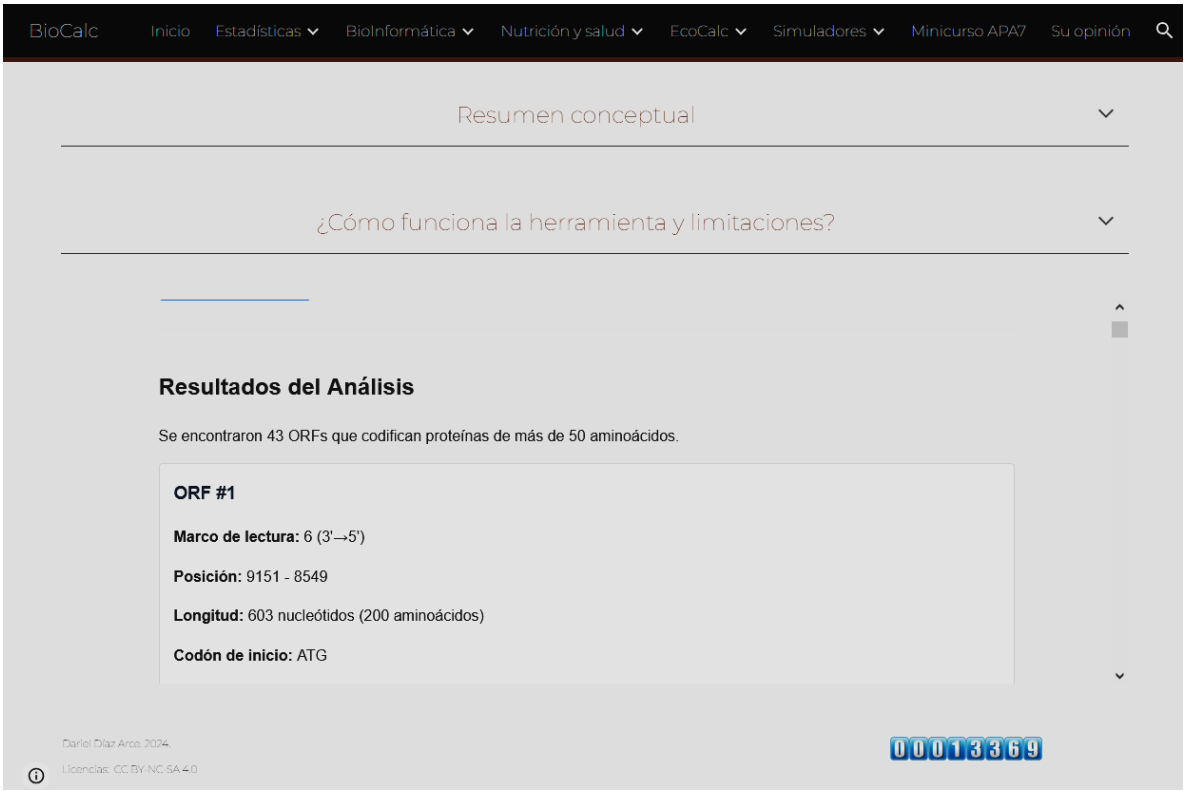


Figure 7. DNA sequence *Aspergillus salvadorensis* to ORF. BioCal 2026

From Figure 7, the analysis of the genetic sequence of *Aspergillus salvadorensis* shows the presence of multiple open reading frames (ORFs), indicating considerable protein-coding potential within the DNA fragment studied. According to the results obtained in the bioinformatics tool, 43 ORFs capable of encoding proteins with a length greater than 50 amino acids were identified, which suggests the existence of several functional regions that could participate in metabolic, structural or virulence processes typical of the fungus.

In particular, ORF #1 represents one of the reading frames detected within the analyzed sequence. This ORF is located in reading frame 6, oriented in the direction of 3' → 5', indicating that it belongs to the complementary strand of DNA. Its genomic position extends from nucleotide 9151 to nucleotide 8549, confirming its inverse orientation within the

sequence. The total length is 603 nucleotides, which corresponds to approximately 200 amino acids after the translation process. This length suggests that it could be a medium-sized protein, potentially involved in specific cellular functions of the body. ORF #1 represents the candidate with the greatest potential for vaccine design within the set of open reading frames identified in the analyzed genetic sequence of *Aspergillus salvadorensis*. This ORF encodes a protein with a length of approximately 200 amino acids, a size that is considered suitable for immunoinformatics studies, as it allows the presence of multiple antigenic regions capable of inducing an immune response. In addition, it has an ATG start codon and a TGA stop codon, indicating a complete and functional reading frame, increasing the probability that it corresponds to a real protein expressed by the organism.

From an immunological point of view, the length of this protein is particularly favorable because it facilitates the identification of different types of immunogenic epitopes, including B cell epitopes, responsible for the production of antibodies, as well as helper T cell epitopes and cytotoxic T cell (CTL) epitopes, which participate in the activation and regulation of the cellular immune response. The presence of these elements is critical for the development of effective vaccines, as a complete immune response requires coordinated activation of both humoral and cellular immunity. In addition, the protein sequence derived from ORF #1 shows a relatively complex structure and no extensive dominant repeats, which favors the structural stability of the protein and increases the probability that it contains functional domains or well-defined antigenic regions. These characteristics are important for subsequent bioinformatic analyses, such as epitope prediction, three-dimensional modeling of the protein, and molecular docking studies with immune system molecules, key steps in the rational development of protein-based vaccines.

Overall, the structural and functional characteristics observed in ORF #1 suggest that this coding region is the best candidate for immunoinformatic studies aimed at vaccine design, since it has favorable properties related to protein length, the integrity of the reading frame, and the immunogenic potential of the protein it encodes. These results provide a solid basis for further analysis, such as the evaluation of antigenicity, allergenicity, toxicity, and the identification of specific epitopes that can be used in the development of vaccine strategies directed against *Aspergillus salvadorensis*.

In addition, the identified ORF has an ATG start codon, which is the most common codon for the initiation of translation in eukaryotic and prokaryotic organisms, since it codes for the amino acid methionine, marking the point where protein synthesis begins. The presence of this start codon increases the probability that this ORF corresponds to a potentially functional gene.

The detection of numerous ORFs in the sequence of *Aspergillus salvadorensis* suggests a complex genetic organization, with several regions capable of producing proteins. These results constitute a first step in the bioinformatic analysis of the genome or genomic fragment of the fungus, since the identified ORFs can subsequently be subjected to homology analysis, functional annotation, structural prediction and immunoinformatic studies, especially relevant when seeking to identify candidate proteins for pathogenicity studies, molecular diagnosis or vaccine development.

Discussion

The results obtained from the structural model indicate that the Gel-type protein analyzed has structural characteristics compatible with the glucanosyltransferases of the GH72 family previously described in filamentous fungi. These enzymes play a central role in the biogenesis and maintenance of the fungal cell wall, particularly by reorganizing β-1, 3-glucan chains that contribute to the mechanical strength of the cell.

The presence of a catalytic domain with barrel-like folding (β/α) 8 (β/α) 8 suggests a conserved catalytic mechanism within this family of enzymes. This type of structural architecture has been described in multipleglycosidic enzymes and is characterized by providing a stable environment for the formation of the active site. The identification of a catalytic residue of aspartate within the enzymatic cavity supports the hypothesis that the analyzed protein could participate in transglycosylation reactions responsible for the elongation of glycan polymers in the cell wall.

The presence of highly accessible hydrophilic surface regions further suggests that the protein could play an important role in the interaction between the fungus and the host's immune system. These exposed regions exhibit structural properties compatible with B-cell epitopes, indicating that they could be recognized by antibodies during infectious processes. In this sense, similar cell wall proteins have been proposed as possible antigenic biomarkers and candidates for the development of antifungal vaccines.

Another relevant aspect is the presence of multiple N-glycosylation sites, which could contribute both to the structural stability of the protein and to its recognition by immunological receptors. Glycans present on the surface of fungal proteins are usually involved in the activation of host defense mechanisms through lectin recognition of immune cells.

The identification of a GPI anchor signal suggests that the protein is localized on the cell surface, which reinforces its possible role in cell wall remodeling processes and in the interaction with the extracellular environment. The accessibility of its catalytic domain and surface epitopes

makes this protein a possible therapeutic target for the development of new antifungal strategies, since inhibition of its activity could compromise the structural integrity of the fungal cell wall.

The genomic and immunoinformatic analysis of the ORF69 protein located in the capsule of *Aspergillus salvadorensis* is a modern approach to identify vaccine candidates against emerging fungal infections. Proteins associated with the cell surface or capsule of microorganisms often play key roles in interaction with the host, including processes of immune system adhesion, colonization, and evasion. Due to their exposure to the extracellular environment, these proteins can be easily recognized by the immune system, making them potential targets for subunit vaccine design. In this context, genomic analysis makes it possible to identify open reading frames (ORFs) that encode proteins with a possible structural or antigenic function, facilitating the selection of candidate antigens using computational tools (Rappuoli, 2000; He & Zhu, 2015).

From an immunoinformatic point of view, the prediction of epitopes within the ORF69 protein represents an essential step to evaluate its immunogenic capacity. Epitopes are specific regions of a protein that can be recognized by antibodies or by T-cell receptors, triggering humoral and cellular immune responses. By using prediction algorithms, it is possible to identify B lymphocyte epitopes and epitopes presented by major histocompatibility complex (MHC) molecules, which allows estimating the immunological potential of a protein without the need for initial laboratory experimentation. This approach has proven useful in accelerating the development of vaccines, especially against pathogens whose experimental characterization is limited (De Groot & Rappuoli, 2004; Flower et al., 2010).

Likewise, the analysis of immunological properties such as antigenicity, allergenicity and toxicity allows the safety and potential efficacy of the identified epitopes to be evaluated. Vaccine candidate proteins must exhibit high antigenicity to induce robust immune responses, but at the same time they must show a low probability of causing allergic reactions or toxic effects in the host. In the case of capsular or surface proteins of fungi of the genus Aspergillus, several studies have shown that these molecules can activate specific immune responses and contribute to the recognition of the pathogen by the human immune system (Latgé & Chamilos, 2019; Wang et al., 2018).

The prediction of the secondary and tertiary structure of ORF69 also provides relevant information on the spatial location of epitopes and their accessibility for immune recognition. Epitopes located in exposed regions of the protein, such as loops or surface domains, are more likely to be recognized by antibodies or antigen-presenting cells. Three-dimensional structural modeling allows the stability of these regions to be evaluated and their possible interaction with immune receptors to be analyzed, which is essential for the rational design of epitope-based vaccines (Patronov & Doytchinova, 2013).

Another important aspect in vaccine design is the evolutionary conservation of epitopes among related species. If certain regions of ORF69 are conserved among different species of the genus *Aspergillus*, a vaccine based on these epitopes could offer cross-protection against multiple fungal pathogens. The identification of conserved regions through comparative and phylogenetic analyses is, therefore, a useful strategy to increase the efficacy and potential coverage of vaccines developed using immunoinformatic approaches (Soria-Guerra et al., 2015).

Despite advances in computational tools, immunoinformatic predictions should be considered as an early stage in the vaccine development process. The identified candidate epitopes and proteins require experimental validation by in vitro and in vivo assays to confirm their immunogenic capacity and safety. In this sense, subsequent studies could include the recombinant expression of the ORF69 protein, the evaluation of its recognition by specific antibodies and immunization tests in experimental models. These stages are essential to determine whether the vaccine candidate is capable of inducing a protective immune response against fungal infections (Rappuoli et al., 2016).

The results of the genomic and immunoinformatic analysis suggest that the ORF69 protein of *Aspergillus salvadorensis* has promising characteristics as a candidate for the design of subunit vaccines. The presence of potentially immunogenic epitopes, their location on the surface of the pathogen and their possible conservation among related species support their biological relevance. However, additional experimental studies are required to validate these predictions and confirm their feasibility in future strategies for preventing fungal infections of the genus Aspergillus.

Conclusion

The final conclusion after the analysis of the **ORFfinder** image is that **ORF69** represents the gene with the greatest biological relevance and immunogenic potential in this fragment of the *Aspergillus salvadorensis genome*. Their identification is not a random finding, but the result of filtering out key evolutionary signals that suggest a structural and defensive function for the fungus.

From a molecular perspective, the fact that this reading frame is the most extensive and is located in a specific chain indicates that the cell invests considerable energy in its transcription. The presence of an initial signal peptide and regions rich in amino acids for glycosylation position it as a **surface protein**, which makes it the face that the fungus presents to the immune system. For a researcher, this ORF is not just a sequence, but the basis for developing new therapeutic strategies, since, being an exposed protein, it is

directly accessible for antibody attack and T lymphocyte activation.

For your students, the pedagogical conclusion is that bioinformatics makes it possible to predict the architecture of an organism before even touching it in a petri dish. **ORF69** serves as the perfect example of how computational analysis can predict with high accuracy the cellular localization and biochemical behavior of a protein. By putting this data together with the immunodiffusion experiment you are planning, students will close the science cycle: from the digital code on the NCBI screen to the physical precipitation reaction in the agarose gel.

In summary, the ORF69 of *Aspergillus salvadorensis* is a high-value candidate that fully justifies its subsequent cloning and experimental characterization, representing a critical step in the study of pathogenesis and immunity to this new specimen.

Gratitude and Recognition

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Conflicts of Interest

The author declares that he has no conflict of interest.

References

1. Adams, D. J. (2004). Fungal cell wall chitinases and glucanases. Microbiology, 150(7), 2029–2035. [https://doi.org/10.1099/mic.0.26980-0] (https://doi.org/10.1099/mic.0.26980-0)
2. BioCal.https://sites.google.com/view/biocalc/bioinform%C3%A1tica/orfs
3. De Groot, A. S., & Rappuoli, R. (2004). Genome-derived vaccines. Expert Review of Vaccines, 3(1), 59–76.
4. Flower, D. R., Macdonald, I. K., Ramakrishnan, K., Davies, M. N., & Doytchinova, I. A. (2010). Computer aided selection of candidate vaccine antigens. Immunome Research, 6(Suppl 2), S1.
5. Free, S. J. (2013). Fungal cell wall organization and biosynthesis. Advances in Genetics, 81, 33–82. https://doi.org/10.1016/B978-0-12-407677-8.00002-6
6. He, Y., & Zhu, B. (2015). Computational vaccinology and the vaccine ontology. Human Vaccines & Immunotherapeutics, 11(2), 287–289.
7. Klis, F. M., Boorsma, A., & De Groot, P. W. J. (2006). Cell wall construction in Saccharomyces cerevisiae. Yeast, 23(3), 185–202.
8. Latgé, J. P., & Beauvais, A. (2014). Functional duality of the fungal cell wall. Current Opinion in Microbiology, 20, 111–117.
9. Latgé, J. P., & Chamilos, G. (2019). Aspergillus fumigatus and aspergillosis in 2019. Clinical Microbiology Reviews, 33(1), e00140-18.
10. Patronov, A., & Doytchinova, I. (2013). T-cell epitope vaccine design by immunoinformatics. Open Biology, 3(1), 120139.
11. Rappuoli, R. (2000). Reverse vaccinology. Current Opinion in Microbiology, 3(5), 445–450.
12. Rappuoli, R., Bottomley, M. J., D'Oro, U., Finco, O., & De Gregorio, E. (2016). Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. Journal of Experimental Medicine, 213(4), 469–481.
13. Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences, 109(16), 6241–6246.
14. Soria-Guerra, R. E., Nieto-Gomez, R., Govea-Alonso, D. O., & Rosales-Mendoza, S. (2015). An overview of bioinformatics tools for epitope prediction: Implications on vaccine development. Journal of Biomedical Informatics, 53, 405–414.
15. Vásquez Hidalgo, A. (2025). Characterization of *Aspergillus salvadorensis* Isolated from Caeselpinia coriaria Seed, El Salvador. IKR Journal of Agriculture and Biosciences (IKRJAB), 1(4), 189-205. https://ikrpublishers.com/wp-content/uploads/2025/11/IKRJAB19233-2025.pdf
16. Wang, T., Houbraken, J., Groenewald, J. Z., Meijer, M., Andersen, B., Nielsen, K. F., & Samson, R. A. (2018). Diversity and taxonomy of the genus *Aspergillus*. *Studies in Mycology*, *91*, 141–173.
17. Yassiba, K., et al. (2022). Structural and functional analysis of GH72 family glucanosyltransferases involved in fungal cell wall remodeling. *Journal of Fungal Biology*, 126(4), 245–258.
18. Yin, Q. Y., de Groot, P. W., Dekker, H. L., de Jong, L., Klis, F. M., & de Koster, C. G. (2008). Comprehensive proteomic analysis of Saccharomyces cerevisiae cell walls. Molecular & Cellular Proteomics, 7(4), 713–723.