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Prevalence of Secondary Metabolites Target Carcinogenic Clusters in the Circular DNA Sequence of *Aspergillus salvadorensis* to Aflatoxins

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Abstract:

The aim of this study was to determine the prevalence of precursor cancer cluster genes in the species *A. salvadorensis*, using the database sequenced by MACROGEN INC Kore del Sur by the circular DNA sequencing method by Illumina of the Metagenome Shotgun Sequencing (NGS) method design of 67M spots, 9.9G bases and 4.3Gb. The following method was used: MetaPhlAn4, where all the genetic information that the identified microorganisms may have was collected through a validated database and for the genes that are not in the database it was translated into protein sequences and compared with a protein database such as UniRef90, although they do not identify anticancer molecules, only precursor genes, this is done by laboratory methods such as HPLC or ELISA. Being carried out in the microbiology laboratories of the Faculty of Medicine and in MACROGEN INC of South Korea. Bioinformatics programs BLAST, CLUSTAL, GENBANK, M11, UGENE, T-COFEE including PYTHON were used. Human subjects were not used in the research. The seed lots were subjected to an ultraviolet (UV) light analysis. This qualitative, simple and fast laboratory method served as a first approximation to identify the possible presence of Aflatoxins which was later confirmed with gene analysis in sequencing. The analysis focused on searching for key genes within the aflatoxin cluster, such as aflC, aflD, aflR and aflS. In our study, the prevalence in the circular DNA chain positive for aflatoxins was negative and if it is negative, does not refer to toxicity, the prevalence would be zero. The use of ultraviolet light to determine aflatoxins is suggestive to find sufficient quantities present in the seeds, possibly used to identify contaminants to fungi. Of the most common species of the genus *Aspegillus* that produce aflatoxins are: *A. flavus, A. pararasiticus, A. nomius, A. ochraceus, A. wentii* in optimal conditions of temperature and humidity. The species *A. salvadorensis* does not have the capacity to produce aflatoxins in toxic doses and does not present carcinogenic aflatoxins according to the report of Macrogen Inc.2025 "gene family UniRef90_M2YIY2: Dothistromin biosynthesis regulatory protein aflR, UniRef90_O42716: Aflatoxin cluster transcriptional coactivator aflS genes related to aflatoxin were not detected". The prevalence is zero. Aflatoxins are chemical toxins produced by certain fungi, not by genetic sequences. What the report refers to is that no toxins were found in the sequence. Therefore, if the genes are missing, the fungus cannot produce the toxin.

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[1]Validation Fungal Name FN 573057, GenBank ID PRJNA1306032

INTRODUCTION

The production of aflatoxins in the aforementioned fungi worldwide is regulated by a set of genes, which are located in a chromosomal region known as the "aflatoxin biosynthetic gene cluster" (AF cluster). Generally speaking, studies of this type focus on identifying the presence of these genes in the DNA of *Aspergillus samples*. The word “aflatox” comes from the suffix and prefix of a = *Aspergillus*, fla = *flavus* and toxin = poison. Aflatoxins are secondary metabolites that chemically correspond to bis-dihydro-furanocoumarins. They were discovered in Great Britain in 1960 (Mollay *et al.*, 2022; Setlem and Ramlal, 2022). Mycotoxins, harmful substances, are mainly generated by two types of filamentous fungi: *Aspergillus and Penicillium*. Of the described species such as *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus oryzae* are recognized for producing these toxins (Bolet Astoviza and Socarrás Suárez, 2005). These fungi often contaminate common foods, especially stored cereals and grains. To produce mycotoxins optimally, specific conditions are needed, such as: an ambient temperature of 25 °C and a relative humidity of 95 %. Eighteen types of these mycotoxins have been identified to date, including B1, B2, G1, G2, M1 and M. 2 of these, aflatoxin B1 (AFB1) is the most significant toxic metabolite of this group E (Bolet Astoviza and Socarrás Suárez, 2005; Carvajal, 2013; Chain *et al.*, 2020), frequently contaminates foods stored in tropical and subtropical regions, such as maize, peanuts, and rice. These mycotoxins are known to be potent foodborne carcinogens and are directly involved in the development of hepatocellular carcinoma. Aflatoxin (AF) contamination in crops represents economic losses of millions of dollars globally. The FAO has estimated that up to 25% of all crops in the world are affected by FA, with cereals, oilseeds and spices being the most impacted (Adhikari *et al.*, 2016; Carvajal, 2013). When animal feed contains aflatoxin levels ranging from 60 to 800 mg per kilogram, this has serious repercussions on animal production. A decrease in production is observed, as well as symptoms such as diarrhea, vomiting and abortions in cattle and cattle. In addition, the presence of these toxins can negate the effectiveness of vaccines in livestock, further complicating animal health and handling (Jallow *et al.*, 2021). Carcinogenic gene clusters in molecular biology refer to groups of genes that are involved in processes that could contribute to the development of cancer if their expression or function is altered. These genes are associated with both nuclear and mitochondrial genomics. However, most studies on cancer-related gene clusters focus on linear (nuclear DNA) and circular (mitochondria) nuclear genomes. Possible clusters would be aflA and aflJ: They encode a series of enzymes responsible for the transformation of the initial aflatoxin precursors, called aflB, aflC, aflD, aflE, aflG, aflI: These genes encode other enzymes involved in the subsequent steps of aflatoxin biosynthesis, such as the conversion of precursors into intermediate compounds that finally give rise to AFB1 aflatoxins, AFB2, AFG1, AFG2, among others (Caceres *et al.*, 2020; Cary *et al.*, 2006; Chalco Quezada, 2014; Mollay *et al.*, 2022; Rojas Jaimes *et al.*, 2021; Yu *et al.*, 2004).

There is a lot of information in the literature on aflatoxins of the genus *Aspergillus* in cereals, but their presence has not been described on *Caesalpinia coriaria* if it has carcinogenic metabolites. It is observed that in the northern area of Morazán and Chalatenango cattle are fed with this seed in feed or feed on the seeds fallen from the tree on the ground where they graze, as well as this in areas of land in arid land or are used as a division fence in wastelands by farmers regularly contaminating the land with the spores of the fungus. Its research is necessary to evaluate, recommend and prevent farmers in the area about the use of *Caeselpinia coriaria* as feed in livestock feed, as well as to carry out prevention campaigns at the national level because there may be cross-reaction from animal to human as a mode of indirect transmission in human health (Caceres *et al.*, 2020). The objective of this study was to

determine the prevalence of precursor cancer cluster genes in the species A. *salvadorensis*.

MATERIAL AND METHODS

Materials

For the development of the research, batches of seeds of *Caesalpinia coriaria* collected in the northern area of the department of Morazán, El Salvador, which are used directly or indirectly in livestock feed, were used. As part of the preliminary analysis, an ultraviolet light source was used to observe the fluorescence of the samples. In the molecular area, we worked with circular DNA sequences of the fungus *Aspergillus salvadorensis*, obtained from databases generated in South Korea through next-generation sequencing platforms and registered in GenBank under the PRJNA1303219 and PRJNA1306032 bioprojects. For the bioinformatics analysis, different specialized programs and databases were used, including BLAST, CLUSTAL, GenBank, M12, UGENE, T-COFEE, MetaPhlAn4, the UniRef90 database and Aspergillus Gene Database, as well as Python scripts. The mushroom culture was carried out on Sabouraud agar and the assembly and annotation of the genome was carried out with the SPAdes, Velvet, Canu, Prokka and AUGUSTUS programs. The sequencing of the genetic material was carried out on platforms compatible with Illumina, PacBio and Oxford Nanopore.

Methods

A descriptive and cross-sectional study was carried out, carried out between February and September in the microbiology laboratories of the Faculty of Medicine, with the purpose of detecting the possible presence of aflatoxins in seeds of *Caesalpinia coriaria*. Initially, the samples were subjected to observation under ultraviolet light as a qualitative, simple and fast method, which allowed identifying indications of possible aflatoxin contamination and selecting the suspicious samples for subsequent analysis. In addition, circular DNA sequences of *Aspergillus salvadorensis* from the GenBank database were analyzed, focusing the study on the search for genes belonging to the biosynthetic cluster of aflatoxins, specifically *aflC*, *aflD*, *aflR* and *aflS*. The fungus was grown on Sabouraud agar under optimal growth conditions and then the extraction of genomic DNA was carried out using a standard protocol, ensuring the obtaining of a high-quality genetic material free of contaminants. In MACROGEN INC (Inc, 2025), the isolated DNA was subjected to a process of enzymatic fragmentation or ultrasound, and in the case of circular DNA, it was previously amplified. Then, genomic libraries were built by adding specific adapters for sequencing on next-generation platforms. The readings obtained were assembled with specialized programs, generating longer continuous sequences and reconstructing the genome of the microorganism. Subsequently, the functional annotation of the genome was carried out, identifying genes, regulatory sequences and the general organization of the genetic material. The presence or absence of the aflatoxin cluster was determined in each isolate analyzed and, based on these results, the prevalence was calculated using the relationship between the number of positive isolates and the total number of isolates analyzed, expressed as a percentage. Additionally, the methodology proposed by MACROGEN INC. was used, which includes metagenomic tools for the identification of microorganisms and the functional prediction of genes. Those sequences that did not present direct match in the DNA databases were translated into protein sequences and compared with the UniRef90 database, which allowed to increase the reliability of the interpretation of the results. Finally, from an ethical point of view, the study did not involve the participation of humans or animals, being limited exclusively to the analysis of plant material and genetic information of the fungus *Aspergillus salvadorensis*, respecting the principles of scientific research (Faroni and Leda, 1993;

Illumina, 2025; Kanehisa and Sato, 2020; Khaldi *et al.*, 2010; Vásquez, 2025a; Vásquez, 2025b).

RESULTS AND DISCUSSION

In photo 1, at the top of the image are four almost identical photographs of the same blue tube or jar placed on a wooden surface. Behind the bottle is a lamp that emits ultraviolet light, which illuminates the edge of the tube and casts a violet glow on the bottom area. Each of the four photos appears to capture the same object under very similar conditions, with slight variations in angle or light intensity. At the bottom of the image there is a black box showing two microscopic photographs taken under ultraviolet light at an indicated magnification of 100x, which are spores of the genus Aspergillus in ultraviolet light at 100x. On the left are shown three rounded structures with an intense blue-white glow of *Aspergillus niger*". On the right is another bright and diffuse structure of *Aspergillus uesalvadorensis.* This indicates that there is a subjective presence of aflatoxins when the nacascol seeds are macerated with alcohol in the presence of ultraviolet light.

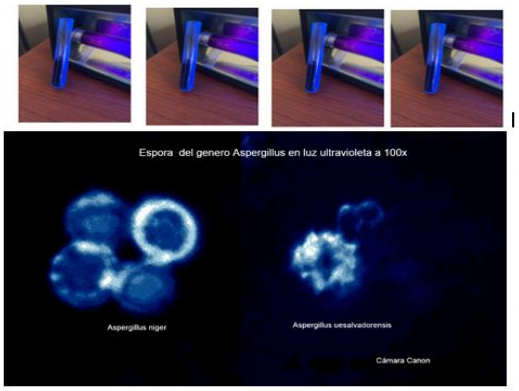


Photo 1. Ultraviolet light exposure from nacascol seeds. 2025

The main carcinogenic metabolite match between *Aspergillus neoniger and Aspergillus salvadorensis* probably includes aflatoxins, although both species are not the most common producers of aflatoxins. Other relevant metabolites include ochratoxins, fumonisins, and patulin. The prevalence of clusters of carcinogenic metabolites in the circular DNA sequence of *Aspergillus salvadorensis* suggests that their impact on animals could be greater than on humans, since humans, through their diet, do not directly consume the seeds of *Caesalpinia coriaria* (Vásquez, 2025b). However, there could be cross-contamination between the animal consuming the contaminated seed and the human being, when consuming meat or milk contaminated with aflatoxins. Although *Aspergillus salvadorensis* is not one of the species best known for producing aflatoxins, it is known that some strains can synthesize them under certain conditions. Genes that could be involved in this production include Genes related to aflatoxin biosynthesis: aflR: It is a key regulator that activates the expression of genes involved in aflatoxin biosynthesis. aflD and aflM: Encode enzymes critical for the conversion of intermediates in the biosynthetic pathway of aflatoxins. aflJ: Encodes an oxidoreductase involved in the final stages of aflatoxin biosynthesis. Ochratoxin biosynthesis genes: Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin, produced in some species of Aspergillus, including strains of *A. salvadorensis*. otap: The gene otap encodes the enzyme ochratoxin A polyketide synthase, which is essential for the biosynthesis of OTA. ochA and ochB: Genes involved in the conversion of precursors to ochratoxin A. Genes for fumonisin biosynthesis: Although fumonisins are most commonly produced by Fusarium species, some strains of Aspergillus can also produce them. The genes involved are: FumA, FumB and FumC: They encode enzymes that participate in the biosynthesis of fumonisins, especially in the structural modification of the toxin. Patulin biosynthesis genes: Patulin is another mycotoxin with genotoxic effects that may contribute to cancer risk. patA: Encodes a key enzyme in patulin biosynthesis. patB and patC: Other

genes involved in the modification of precursors in the biosynthetic pathway of patulin. General regulators of mycotoxin biosynthesis: LaeA and VeA are master regulators that control the production of secondary metabolites, including aflatoxins and ochratoxins. LaeA regulates the biosynthesis of various toxins, while VeA is involved in the cell cycle, development, and production of toxins, influencing the biosynthesis of aflatoxins and other mycotoxins. Genes related to stress resistance and secondary metabolite production: Fungi produce secondary metabolites in response to stress conditions (nutritional, environmental, etc.). Some relevant genes include nmrA: A regulator that can affect the production of several secondary metabolites. brlA: Regulates spore formation and may be involved in the production of toxins (Adhikari *et al.*, 2016; Casquete *et al.*, 2019; Keller and Hohn, 1997). Although information on *Aspergillus salvadorensis* is more limited compared to other species of the genus Aspergillus, the aforementioned genes and their biosynthetic pathways represent possible candidates for the production of carcinogenic mycotoxins in this species. However, more specific studies are required to confirm the presence and activity of these genes in *A. salvadorensis.* By using the PHYTON program to identifying the precursor codons of ORFs to aflatoxins, it is determined because they are formed by triplets of nitrogenous bases (A, T, C, G) that code for the different amino acids. ATG is the starting codon (for methionine). TAA, TAG, TGA are stop codons. In our case, using BLAST and PHYTON, some of the ORFs found were: ATGATCAAATAA: ATGCATGCAGGAGACTCTGTGAAAGTGCATT GTATATGTAGTTCGAAAATTATTCGGGTTAC CTCTCTCTCCTAA: A long ORF, which begins with ATG and ends with TAA. ATGCAGGAGACTCTGTGA: Another ORF that ends with the TGA stop codon were to identify aflatoxin precursor metabolites, they are: ATGATCAAATAA,ATGCATGCAGGAGACTCTG TGAAAGTGCATTGTATATGTAGTTCGAAAATT ATTCGGGTTACCTCTATCTCCTAA,ATGCAGG AGACTCTGTGA,ATGTAG,ATGCCTGGATTCT AA,ATGTTGACCTGGCCTGCAAAAATGACAG GGAAAGCTACCTTAGATGCTTTGATGTGGTA A,ATGACAGGGAAAGCTACCTTAGATGCTTT GATGTGGTAA,ATGCTTTGA,ATGTGGTAA,AT GTGGGAAATGTTCTCATAG,ATGTTCTCATA. There are 7 metabolites present in these short sequences. The following sequences were found using programs such as BLAST, AUGUST and SnapGene.

In Figures 1, 2 and 3, the BLASTx analysis performed on the sequence of 517 nucleotides reveals significant coincidences with hypothetical proteins of different species of the genus Aspergillus, mainly *A. niger, A. tubingensis and A. sclerotioribanaticus*, all belonging to the clade of the fungi Ascomycetes. The best alignments show identities between 88% and 97%, with a relatively low query cover between 22% and 29%, indicating that the fragment analyzed only covers a partial portion of the entire gene. The extremely low E-values (on the order of 10^{-20} to 10^{-29}) confirm that the coincidences are statistically significant and not the product of chance.

```
>acvA_candidate_Aspergillus_uessalvadorensis_region_8733_9251 length=519
TCATCTCCGTCCATAGCCCCGTTGCTGCAGAGTGACTCGGAAGTAACTGATGTCCTCTTCCGGA
CTCGATATTCGGGACTGCGGCGGCACTAGTTCCGTCAGATCCTAATCATGGCGGCTGAGGCAGA
TCTATCGGAGGGCCGCATGGCCGACATCATCCGTGCCCTTGAGCTCATCCATAGCCCCTCCTCG
ACAAATGAACTACGCAGGGAGGCCTTGACCTTTGAAGGATTCAAGCTTAATCTACGTGCACAGAT
TATCCGACATCGCTCGTAAAATCGTCTCCACCATCGTATCATACCCCCCCAGCATCTGCTGGAAC
CATTCCACCTCCAGTCCGTCCGTCCCATTGAGCTCTCGGAGCTCGGGACTCAGATTTTTGCACAT
TGAGATTGAGGCGTTTTGCAGCCAAGGTGGGTACTTCTGTCTTTACAATGATGACAATGTCGTGA
AATGTTAGGCTGGGGAGCGTTGAAAAAAATCTGTGACGCAAAACTACTTATCCATCTCATTTTTG
```

Fig. 1. Short sequence of *Aspergillus salvadorensis*. 2025.

Fig. 2. Using Blast for the short sequence. BLAST 2025 Font.

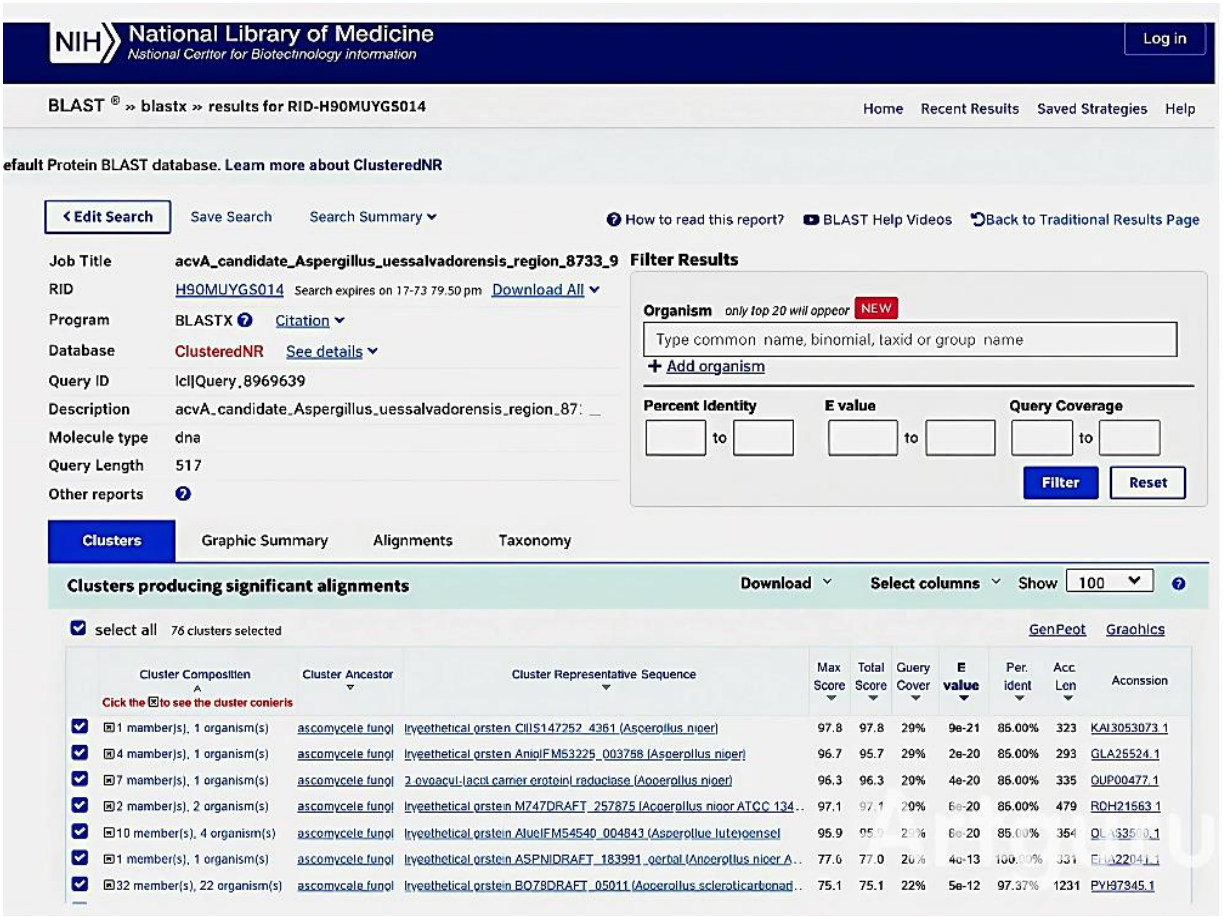


Fig. 3. *Aspergillus salvadorensis sequence*. Blastx. 2025

The first results of the alignment correspond to annotations such as "hypothetical protein" in different species of Aspergillus niger and "3-oxoacyl-(acyl carrier protein) reductase", a metabolic enzyme involved in fatty acid or polyketide synthesis pathways. These

coincidences suggest that the fragment could encode a reductase-like enzyme, which may be associated with a secondary biosynthesis cluster rather than with another function, possibly associated with a secondary biosynthesis cluster, and not with a regulatory factor such as AflR or AflS.

Absence of relationship with AflR/AflS

None of the alignments show similarity to either AflR (aflatoxin regulatory protein) or AflS (AflJ) proteins. Therefore, it is unlikely that the sequence analyzed encodes any of these aflatoxin regulators but rather an enzyme belonging to a different biosynthetic cluster, possibly related to the production of polyketides or other secondary metabolites.

Partial coverage and recommendations

The low coverage (~25–30%) suggests that the 517 nt fragment corresponds only to an incomplete portion of the gene, probably at its 5′ or 3′ ends. To achieve an accurate functional identification, it would be necessary to obtain a high degree of identity with *A. niger, A. tubingensis and A.* sclerotioribanaticus. The sequence belongs to a gene conserved within the *A. niger–tubingensis–salvadorensis* complex, which reinforces its authenticity and probable genomic origin in *Aspergillus salvadorensis*. In summary, the sequence analyzed does not correspond to the regulatory genes AflR or AflS but is associated with a reductase-like enzyme or other hypothetical protein involved in secondary metabolic pathways. The fragment is genuine and conserved among closely related species, although too short for a complete functional characterization. It is likely to be part of a secondary biosynthesis cluster, possibly related to polyketides or non-ribosomal peptides (NRPS), where genes such as ACVA or similar reductases are usually grouped together. When using SnapGene, BANK, and AUGUST 2025, you have: From the long sequence (de Salud, 2002; Keller and Hohn, 1997; Khaldi *et al.*, 2010).

In Figure 4,5 and 6, the sequences for the search for aflatoxin precursor genes have been ordered. In figure 3, the restriction map corresponding to the sequence *Aspergillus salvadorensis* ITS_UES-001_clean, with a total length of 2450 base pairs (bp), represents the distribution of cut sites generated by various restriction enzymes throughout the DNA fragment. At the top of the axis of the map, the names of the enzymes are indicated along with the position (in bp) where each one recognizes and cuts the molecule. These positions refer to the starting point of recognition on the linear sequence. Among the enzymes identified, DraIII (170 bp) and BsaWI (145 bp) stand out, located near the 5′ end, which evidences the presence of early cut sites. In an intermediate zone of the fragment appear SpeI (496 bp), StuI (473 bp) and PfoI (490 bp), which form a group of sites close to each other. In the first third of the sequence, a slight enzyme concentration corresponding to EagI (651 bp) and BssSI/BssSQI (626–651 bp) is also observed. In the central region, the sites of BsrGI (1168 bp), BstXI (1125 bp) stand out. bp) and HpaI (984 bp), reflecting a moderate density of digestion points. Finally, towards the 3′ extreme (after 1500 bp), several relevant enzymes are concentrated, such as AccI (1686 bp), BanII (1809 bp), PstI (1837 bp), TaqII (1882 bp) and ApoI (1921 bp). This accumulation of sites between 1700 and 2100 bp, where DrdI, BsaBI, BbvCI, Bpu101 and SfiI are also found, suggests a region with high potential for cloning analysis or molecular characterization, given its abundance of restriction points. The restriction map is a fundamental tool in molecular biology, as it allows: In this case, the fragment analyzed corresponds to the ITS (Internal Transcribed Spacer) region of the ribosomal DNA of *Aspergillus salvadorensis*, a molecular marker widely used for taxonomic identification and phylogeny of fungi. The length of 2450 bp and the presence of numerous restriction sites reflect a complex sequence that is useful for comparative studies, both evolutionary and molecular diagnostics. The map does not show the presence of ORFs (Open Reading Frames) or coding sequences, which confirms that it is a non-coding fragment. Therefore, the sequence does not contain structural genes or biosynthetic enzymes, and its function is phylogenetic, not

metabolic. The analysis of the restriction line map of the sequence *Aspergillus salvadorensis* ITS_UES-001_clean (2450 bp) shows that it corresponds to a non-coding region of ribosomal DNA (ITS), used as a marker of genetic identification between species of the genus Aspergillus. Consequently, no genes related to the biosynthesis of aflatoxins are detected, since these are found in a different genomic cluster, present only in toxigenic species. Thus, the sequence analysed reveals the genetic identity of the fungus but does not indicate its aflatoxigenic capacity, reaffirming its taxonomic and phylogenetic value rather than its functional or metabolic.

Fig. 4. Long sequence of *Aspergillus salvadorensis*. 2025. It has: A clean and orderly sequence is obtained:

Fig. 5. Clear and orderly sequence 425.663 bp of *Aspergillus salvadorensis*. SnapGene 2025

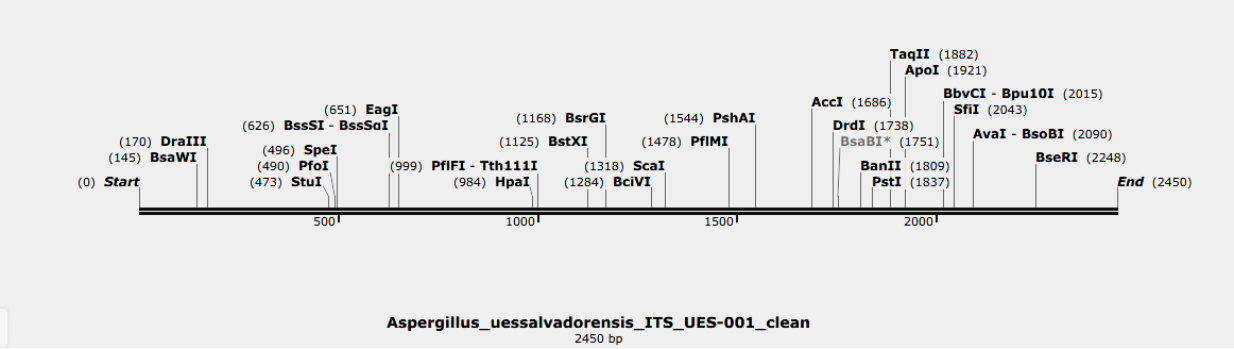


Fig. 6. Linear sequence of *Aspergillus salvadorensis*. SnapGene 2025 Font

In Figure 7, the circular restriction map presented corresponds to the sequence *Aspergillus salvadorensis* ITS_UES-001_clean, with a total length of 2450 base pairs (bp). This type of graphical representation is widely used in molecular biology to visualize the enzymatic cutting sites of a DNA sequence. Although the ITS (Internal Transcribed Spacer) region is linear in nature, the circular projection is especially useful for comparing shear positions and analyzing the global distribution of restriction enzymes more intuitively. Along the circle, the cutting sites of different enzymes are marked, along with their exact position in bp. Start of the map (0–500 bp): the first cut-off sites corresponding to BsaWI (145 bp) and DraIII (170 bp) appear, followed by StuI (473 bp), PfoI (490 bp) and SpeI (496 bp), which make up an area with a high density of early cuts. Middle region (500–1300 bp): EagI (651 bp), BssSI/BssSQI (626 bp), HpaI (984 bp), PflFI-Th111I (999 bp), BstXI (1125 bp) and BsrGI (1168 bp) are observed, showing a clustering of sites in the middle part of the sequence. Posterior zone (1300–2100 bp): ScaI (1284 bp), BciVI (1284 bp), PflMI (1478 bp), PshAI (1544 bp), AccI (1686 bp), and DrdI/BsaBI (1738–1751 bp) stand out, followed by BanII (1809 bp), PstI (1837 bp), TaqII (1882 bp), and ApoI (1921 bp). This region has a high concentration of restriction sites, especially between 1600 and 2100 bp. Extreme 3′ (2000–2450 bp): BbvCI-Bpu101 (2015 bp), SfiI (2043 bp), AvaI-BsoBI (2090 bp) and BseRI (2248 bp) are located, closing the circle at point 2450 bp (End). The circular map reflects the organization and density of restriction sites in the ITS sequence of *Aspergillus salvadorensis*. This type of analysis makes it possible to identify which enzymes cut the sequence and in which positions, key information for various applications in molecular biology, including: The circular representation also facilitates the identification of areas of high enzyme density, such as the one between 1600 and 2100 bp, which could correspond to more variable regions or with repetitive motifs. In contrast, the initial and central regions have fewer cuts, which suggests a greater structural or functional conservation. The circular restriction map of the *Aspergillus salvadorensis* sequence ITS_UES-001_clean (2450 bp) shows a complex but organized distribution of cleavage sites for multiple enzymes, reflecting the structural diversity of the ITS region. This pattern is useful for molecular, comparative, and evolutionary studies within the genus Aspergillus, and is especially valuable as a reference in genomic or taxonomic analyses. It should be noted that this fragment does not contain aflatoxin precursor genes, as it belongs to the ITS region of ribosomal DNA, a non-coding area used for genetic identification, not for the synthesis of secondary metabolites. The genes involved in the production of aflatoxins such as aflR, aflC, aflP or aflQ – are located in an independent biosynthetic cluster, located in other regions of the genome and present only in toxigenic species, such as Aspergillus flavus or *A. parasiticus*. In summary, the ITS sequence analyzed serves as a taxonomic identification marker of *A. salvadorensis* but does not allow inferring its aflatoxigenic potential, since it does

not encode or regulate genes of the biosynthetic pathway of aflatoxins.

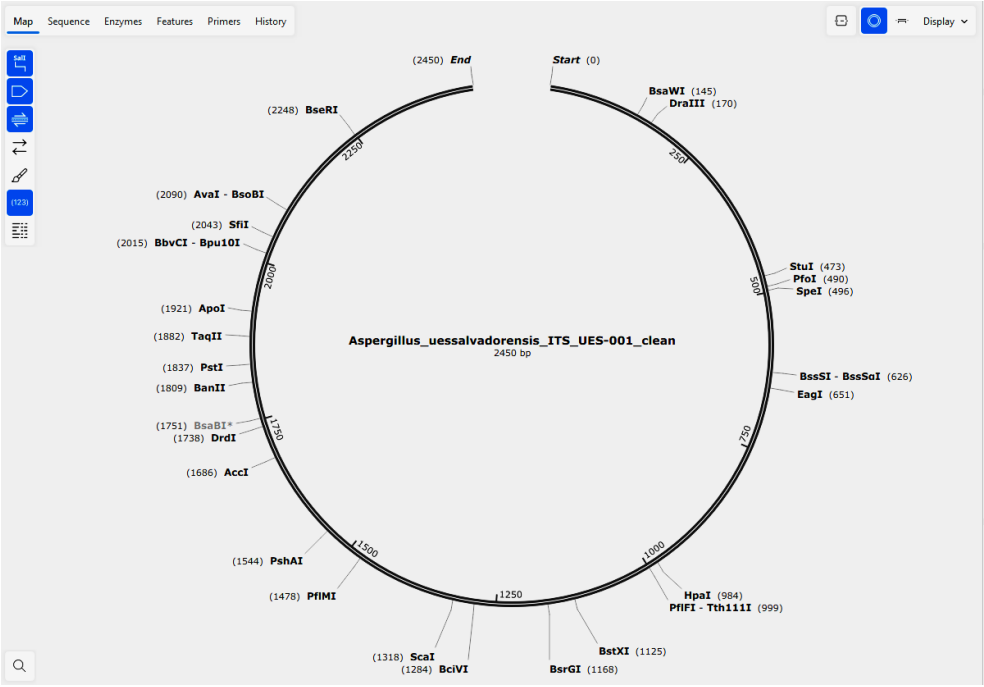
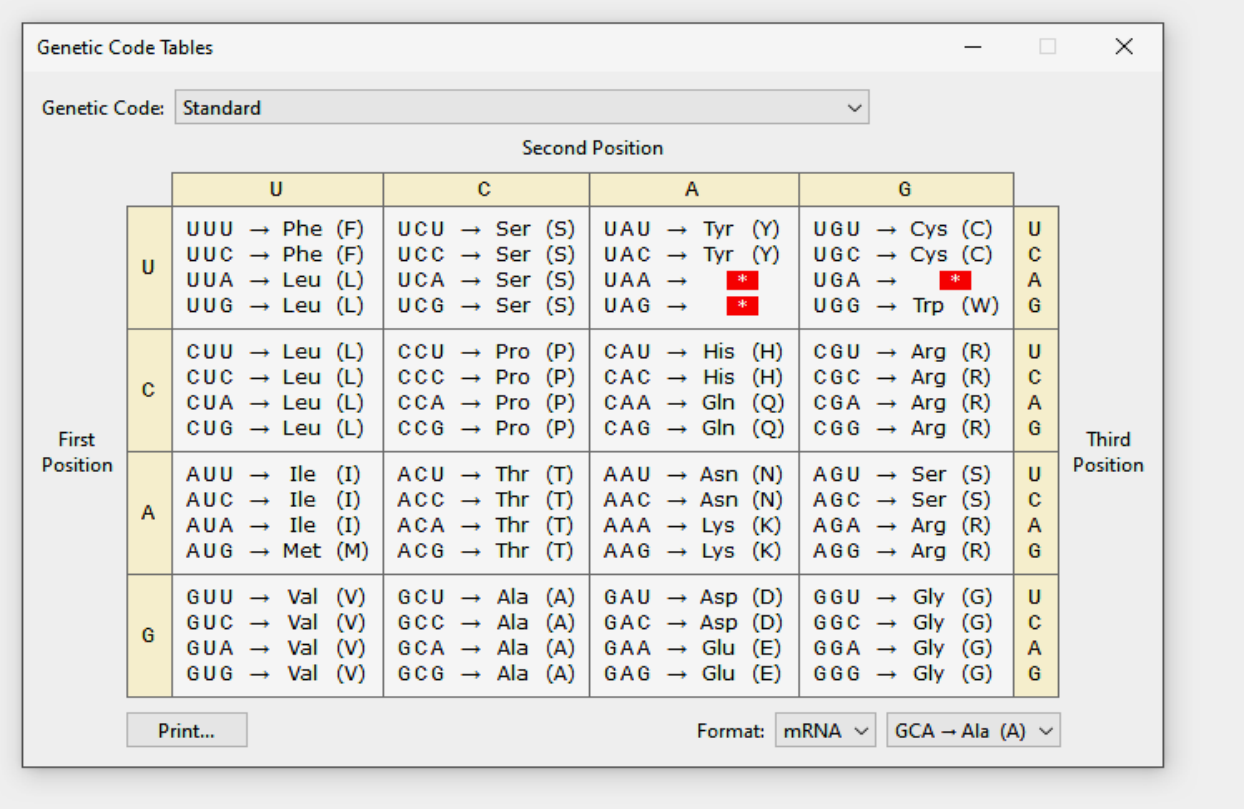


Fig. 7. Circular DNA sequence of *Aspergillus salvadorensis*. SnapGene 2025 Font.

A table of the standard genetic code represents the universal system by which messenger RNA (mRNA) sequences are translated into amino acids during protein synthesis (Table 1). Each set of three consecutive nucleotides—called a codon—corresponds to a specific amino acid or termination signal. The organization of the table is based on the three positions of the codon: The first position is shown in the left sidebar (U, C, A, G). The second position is indicated at the top of the table (U, C, A, L). The third position is arranged in the right row (U, C, A, G). The crossing of these three positions determines the resulting amino acid or, in some cases, a stop signal. For example, the UUU and UUC codons code for phenylalanine (Phe, F), while AUG codes for methionine (Met, M), which also acts as the starting codon of the translation process. The codons UAA, UAG, and UGA function as stop codons, signaling the ribosome the end of protein synthesis. The standard genetic code is virtually universal among organisms, with only slight variations in some mitochondrial genomes or in certain microorganisms. Its main characteristic is redundancy or degeneracy, since multiple codons can encode the same amino acid. For example, the codons GCU, GCC, GCA, and GCG all code for alanine (Ala, A). This redundancy confers evolutionary robustness, since point mutations in the third position of the codon (known as the synonymous position) do not necessarily alter the resulting amino acid, thus reducing the impact of genetic errors and contributing to the stability of the translation system. Aflatoxins are potent and carcinogenic mycotoxins, the product of a complex secondary metabolic pathway, controlled by a coordinated group of genes known as the afl cluster. This genomic cluster, approximately 70–75 kilobases, contains 25–30 genes encoding enzymes, regulatory and transporter proteins involved in the transformation of simple precursors such as acetyl-CoA into highly toxic compounds, such as aflatoxins B_1, B_2, G_1, and G_2. The biosynthetic process begins with the action of a polyketide synthase, encoded by the aflC gene (also called pksA), which forms the initial polyketide skeleton.

Table 1. Genetic code of protein synthesis of *Aspergillus salvadorensis*. SnapGene 2025 Font.



From this point, a sequence of reactions catalyzed by oxidoreductase, dehydrogenases, monooxygenases, and methyltransferases enzymes—encoded by genes such as aflD (nor-1), aflM (ver-1), aflP (omtA), and aflQ (ordA)—generates intermediate metabolites such as norsolorinic acid, versicolorin A, sterigmatocystin, and, finally, active aflatoxins. The aflR encodes a transcription factor responsible for activating the expression of biosynthetic genes by binding to their promoter regions. aflS (also called aflJ), which acts as a cofactor and enhances the function of AflR, increasing transcription and toxin production. The result is a highly coordinated enzymatic and regulatory network, the end product of which is toxic compounds that the fungus can release into the environment. From a biological perspective, aflatoxins thought to function as defense or competition mechanisms, giving the fungus an adaptive advantage over other microorganisms or adverse environmental conditions. Aflatoxins are not isolated products but the result of a complex biosynthetic chain regulated by a cluster of interdependent genes. The precursor proteins involved—primarily polyketide synthases, oxidoreductases, and methyltransferases—convert simple metabolites into highly elaborated toxins. The afl cluster is one of the most detailed and studied examples of genetic organization of a secondary metabolic pathway in fungi, and its understanding has been key both for basic molecular biology and for the development of biotechnological strategies aimed at mycotoxin control and food safety. Therefore, the programming method for the study of MACROGEN clusters uses advanced methods such as MetaPhlAn4 and they are compared with a protein database such as UniRef90. MetaPhlAn4 and UniRef90 bioinformatics programs do not detect carcinogenic aflatoxins as they are chemical molecules. Instead, its function is to identify the genetic components of the producing fungi in a

sample, allowing the risk of contamination to be inferred. The MetaPhlAn4 method, this tool determines the presence and abundance of potential fungi with risk to human health by analyzing DNA sequences and compares them with marker gene databases to identify key species, such as *Aspergillus flavus* and *Aspergillus parasiticus*. The simple detection of these fungi already indicates a potential risk, since they are the organisms capable of generating the mycotoxin. Instead, UniRef90 focuses on determining which avenues use them. Aflatoxins are produced by a specific metabolic pathway controlled by genes (such as *AFLR* and *AFLS)* to seek out the proteins/enzymes that encode these genes. If the sequences of the sample match these proteins of the biosynthesis pathway, it is confirmed that the *detected Aspergillus* is not just any strain, but a toxigenic strain with the genetic capacity to produce the carcinogenic toxin. Together, MetaPhlAn4 identifies the organism at risk and UniRef90 confirms whether the organism possesses the *molecular machinery* to manufacture the aflatoxins, providing an in-depth assessment of the risk in the sample. According to the MACROGEN report, no genes related to aflatoxin production were found in *Aspergillus salvadorensis*, and it was indicated that genes associated with aflatoxin biosynthesis, such as aflR and aflS, were not detected in this analysis: "The analysis results indicated that gene family UniRef90_M2YIY2: Dothistromin biosynthesis regulatory protein aflR, UniRef90_O42716: Aflatoxin cluster transcriptional coactivator aflS genes related to aflatoxin were not detected" (Leiva Rivas *et al.*, 2023). Therefore, the prevalence is zero; there are no specific aflatoxin-producing genes that compromise human health.

In Figure (8), a phylogenetic analysis places the evaluated sequence within the genus *Aspergillus*, in the group formed by the species *A. niger*, *A. tubingensis*, *A. luchuensis*, *A. costaricensis*, *A. piperis* and *A. neoniger*. In the tree obtained by the *Fast Minimum Evolution* method, the sequence identified as **Query_22507** is positioned in the same clade as *Aspergillus tubingensis*, which shows a close genetic relationship between the two. The short distances between branches indicate a high genetic similarity between the analyzed sample and the species belonging to the A*. niger/tubingensis complex*.

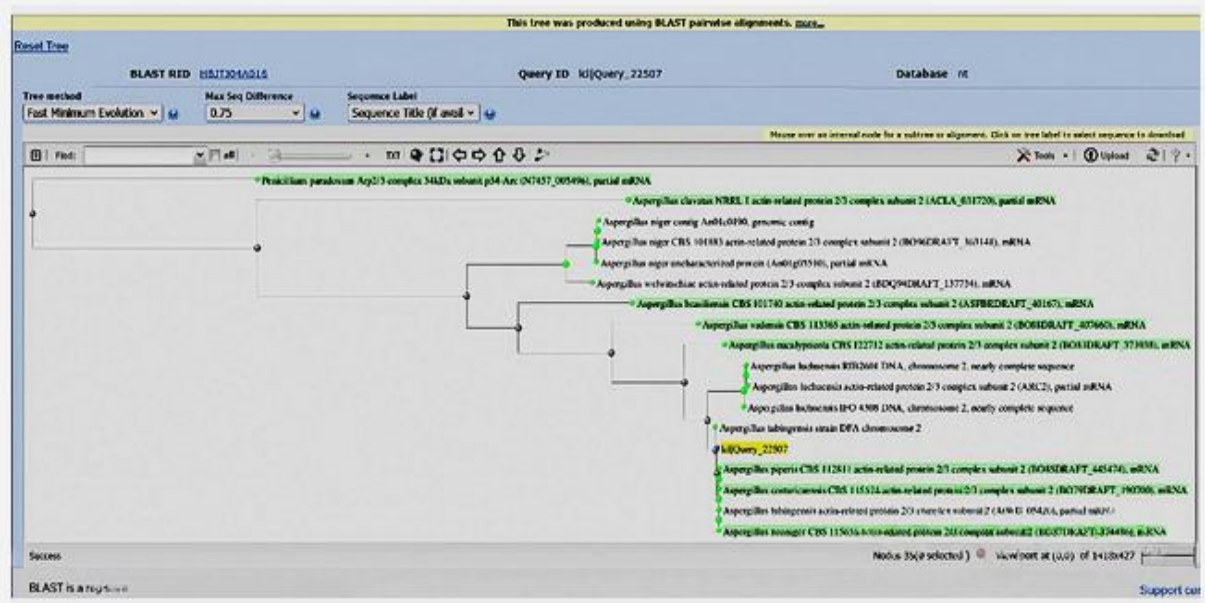


Fig. 8. Arbol filogenético de la secuencia de la tabla 3. Blast. 2025

On the other hand, more distant species, such as *Penicillium paradoxum*, are located outside this group and act as an external reference. The results of the BLAST analysis, which showed 100% identity and very low E-values, confirm the correspondence of the sequence with organisms of the genus *Aspergillus*, especially with members of the A. *niger* complex. Overall, the phylogenetic and molecular similarity data support that the sequence analyzed belongs to ***Aspergillus salvadorensis***, a species phylogenetically related to *A. tubingensis* and *A. luchuensis*, with which it shares genetic homology.

When comparing the genomic organization in the evolutionary degeneration or modification of the gene cluster of the aflatoxinas, we have the *Aspergillus salvadorensis* with highly toxigenic species such as *A. flavus* and *A. parasiticus*.

In Figure (9), based on the phylogenetic tree obtained from the three sequences analyzed, it is observed that *Aspergillus_salvadorensis* (UES-001, complete ITS) is separated from the other two sequences with a relatively high genetic distance.

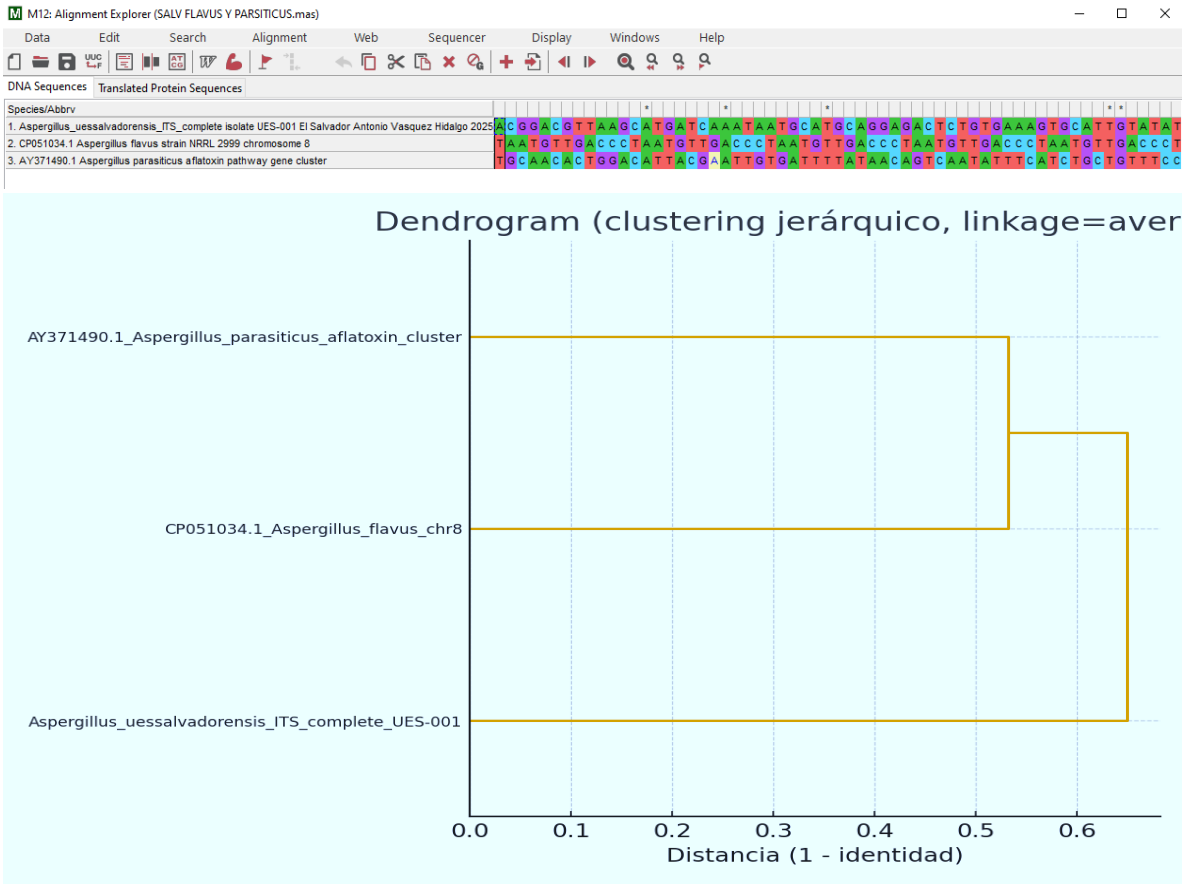


Fig 9. Comparison of sequences of *Aspergillus salvadorensis, flavus and parasiticus*. MEGA 12.2025

The calculated distances, expressed as *1 – identity*, range from 0.53 to 0.67, indicating a low level of direct similarity between the sequences compared. Within the group formed by *Aspergillus flavus* (chromosome 8) and *Aspergillus parasiticus* (cluster of genes of the aflatoxin pathway), a greater relative closeness between them is observed (distance ≈ 0.53)

compared to A. salvadorensis, which has greater distances from both (≈ 0.63–0.67). This relationship is coherent from a biological point of view, since *A. flavus* and *A. parasiticus* are closely related within the genus *Aspergillus*, particularly within the *Flavi group*, associated with the production of aflatoxins. On the other hand, the sequence corresponding to *A. salvadorensis* appears as the most divergent in this analysis, forming a separate branch of the cluster that groups *A. flavus* and *A. parasiticus*. This suggests that isolation represents a genetically distinct entity with respect to the species compared in this preliminary study. It is important to note that the comparison was made between non-equivalent genomic regions: an ITS sequence (ribosomal region used for fungal identification) was compared against a complete chromosomal fragment and a specific gene cluster. This difference in the nature and length of the sequences can artificially increase the observed genetic distances and, therefore, the tree obtained should be interpreted as exploratory and not definitive.

Aspergillus can proliferate in humid and poorly ventilated environments, regular cleaning is necessary and the maintenance of air filters, ducts and humidifiers helps to reduce the presence of *Aspergillus* spores in closed spaces. Likewise, controlling humidity levels and reducing excess humidity helps prevent their growth. In the agricultural field, it is important to apply safe practices during the cultivation, storage and processing of the products. This involves monitoring and regulating humidity levels, as well as ensuring adequate storage conditions to avoid *Aspergillus* contamination. Establishing and enforcing quality controls on a consistent basis can significantly decrease the presence of mycotoxins in food, which in turn reduces the risk of developing cancer. Informing the population about the potential risks and promoting preventive practices can help reduce cases of cancer related to this fungus. It is important for healthcare professionals to educate their patients especially those with weakened immune systems or respiratory conditions about the need to avoid environments that are conducive to the proliferation of *Aspergillus* (Illumina, 2025). In our case, no change it genes were detected for lung or gastric cancer. Some *Aspergillus* of the flavus species have been shown to have aflatoxins of the aflR type (aflatoxins, otap, ochA, ochB (ochratoxins), patA, patB, patC (patulin), aflD, aflM (aflatoxins), fumA, fumB, fumC (fumonisins), LaeA and VeA (general regulation of toxins) that are carcinogenic in humans. In summary, the fungi of the genus Aspergillus that are most involved in the production of carcinogenic aflatoxins are *Aspergillus flavus*, *Aspergillus parasiticus*, and to a lesser extent, *Aspergillus nomius*. The A. *flavus* is the species that contaminates food most frequently. These toxigenic species are capable of generating several types of these mycotoxins, including B1, B2, G1 and G2. Of all of them, Aflatoxin B1 (AFB1) is the most worrying. This is because it is the most potent and has been classified as a Group 1 carcinogen (definitely carcinogenic to humans) by IARC, as it is strongly linked to the development of liver cancer. Both species of fungi are capable of producing a variety of secondary metabolites, some of which may be toxic or carcinogenic. Common carcinogenic metabolites among these species are mainly related to mycotoxins, toxic compounds produced by fungi that negatively affect human and animal health. The most relevant metabolites in terms of their carcinogenic potential that could coincide between *A. neoniger and A. salvadorensis* are the following: Aflatoxins: These are a group of toxic and carcinogenic compounds produced by some species of *Aspergillus*, particularly *A. flavus and A. parasiticus*. *Although A. neoniger and A. salvadorensis* are not the main producers of aflatoxins, under the right conditions they could generate them in low quantities.

Aflatoxins are known carcinogens, especially linked to liver cancer. Ochratoxins: Ochratoxin A (OTA) is another secondary metabolite that exhibits toxic and carcinogenic effects, and has been associated with kidney damage. IARC classifies it as a possible human carcinogen (Group 2B). *A. salvadorensis* may produce

ochratoxins under certain conditions, and A. neoniger has also been reported to produce this mycotoxin, although not as frequently as other Aspergillus species. Fumonisins: Usually associated with species such as Fusarium, some strains of *Aspergillus* can also produce fumonisins. These mycotoxins have neurotoxic and hepatotoxic effects, and are considered carcinogenic, particularly in relation to esophageal cancer. Although *A. neoniger and A. salvadorensis* are not known as large producers of fumonisins, they could generate them in small amounts depending on environmental conditions. In summary, although they are not highlighted as the main producers of these metabolites, both species of Aspergillus may share the ability to produce several carcinogenic metabolites under specific environmental conditions (Carvajal, 2013). Patulin is another mycotoxin that has been reported in some species of Aspergillus. Although not classified as a direct carcinogen, it has been observed that it can have genotoxic effects in animal models, which may increase the risk of cancer in the long term. As for the genes involved in the biosynthesis of aflatoxins, species such as *Aspergillus salvadorensis* possess a series of genes such as aflR, aflM, aflK, aflP, VeA, aflD, aflJ, aflQ, LaeA and AflS. These genes constitute a complex network of regulators and biosynthesizers; however, A. *salvadorensis* does not have the necessary mechanisms to synthesize aflatoxins, unlike other species of the genus Aspergillus, which are capable of producing aflatoxins, highly carcinogenic compounds that are dangerous to human and animal health (Jallow *et al.*, 2021; Kanehisa and Sato, 2020).

The expression of these genes can be influenced by environmental factors such as temperature, humidity and nutrient availability, all of which are key determinants in the production of aflatoxins in these fungi. The genus *Aspergillus* includes several species known for their ability to generate aflatoxins, which are potent and carcinogenic mycotoxins. Aflatoxins occur mainly under stress conditions, such as high temperatures, high humidity and poor growing conditions. Below is a list of the most recognized Aspergillus species for their ability to produce aflatoxins: *Aspergillus flavus*: This is probably the most well-known and studied species in relation to the production of aflatoxins. It mainly produces aflatoxins B1 and B2, which are common in grains such as corn and peanuts, among other agricultural products. Optimal conditions: A. flavus generates aflatoxins under conditions of high temperature (30-37°C) and humidity. *Aspergillus parasiticus*: Another keystone species in the production of aflatoxins, especially aflatoxins B1, B2, G1 and G2. Aflatoxins G1 and G2 are less common and are found mainly in A. parasiticus. Optimal conditions: Like A. flavus, A. parasiticus produces aflatoxins under warm temperatures and high humidity. *Aspergillus nonius*: This species is also associated with the production of aflatoxins, although to a lesser extent than *A. flavus and A. parasiticus* (Inc, 2025; Keller and Hohn, 1997; Khaldi *et al.*, 2010; Mollay *et al.*, 2022). In summary, although *Aspergillus salvadorensis* does not possess the genes necessary to produce aflatoxins, other Aspergillus species such as A. flavus and A. parasiticus are known for their ability to generate these dangerous toxins under specific conditions of environmental stress. Aspergillus nomius is a less common but relevant species, as it also has the ability to produce aflatoxins, especially aflatoxins B1 and B2. It is mainly found in nuts, such as pistachios and almonds.

Like other species in the genus *Aspergillus, A. nomius* thrives in warm, humid conditions. *Aspergillus tamarii*, although less frequent, also has the ability to produce aflatoxins B1 and B2. It is found mainly in cereals and seeds. Optimal conditions: This species, like many others in the genus, grows best in warm, humid environments. *Aspergillus pseudotamarii* is another species that can produce aflatoxin B1 and B2, although not as commonly as A. flavus or A. parasiticus. It has been found in food products such as cereals and seeds. *Aspergillus salvadorensis*, although not one of the most recognized species for its production of aflatoxins, some studies suggest that it can

synthesize aflatoxins B1 and B2 under certain stress conditions. This species has been little researched in terms of its ability to produce mycotoxins, but its potential to generate aflatoxins depends on specific environmental factors, making it less predictable than other more common species*. Aspergillus ochraceus*, best known for the production of ochratoxins, also has the ability to produce aflatoxins in some strains under certain conditions, although aflatoxins are not its main secondary metabolite. *Aspergillus wentii*, although a less common species, some strains of *A. wentii* can also produce aflatoxins B1 and B2. The production of aflatoxins typically occurs in conditions of warm temperatures (between 25-37°C) and high humidity. The presence of decomposing organic matter, such as grains, nuts and other foods, also favors the colonization of these fungi and the production of toxins. As for cancer precursor genes, although *Aspergillus salvadorensis* is not one of the species most commonly associated with the production of aflatoxins, its ability to synthesize them indicates that the genes involved in the biosynthesis pathway could be similar to those present in A. *flavus and A. parasiticus*. Genes directly involved in aflatoxin biosynthesis in Aspergillus include: aflR is the major positive regulator of aflatoxin biosynthesis. Its function is to act as a transcription factor that activates the expression of several other genes in the biosynthetic pathway. This gene is essential for the production of aflatoxins, since, without its presence, the synthesis of these toxins does not occur. naflD´s function: It encodes a polychetide synthase (PKS), an enzyme essential in the early stages of aflatoxin biosynthesis. This enzyme is responsible for forming the intermediate aflatoxycol, a critical precursor in the production of aflatoxins. Its function is key to transforming precursors into the first important compound in the biosynthetic pathway. aflM´s function: It encodes a CYP450 monooxygenase, which participates in the modification of intermediate compounds during the biosynthesis of aflatoxins. This gene is essential for the hydroxylation of precursors, a critical step in converting them to aflatoxins in their final form. aflJ´s function: It encodes an oxidoreductase enzyme that participates in the final stages of aflatoxin biosynthesis, transforming intermediates into aflatoxins. aflK´s function: It encodes a transferase, an enzyme that facilitates the conversion of precursors into aflatoxins. Their role is crucial in the completion of the synthesis of aflatoxins from the intermediate compounds generated by other genes. aflQ´s function: aflQ acts as a positive regulator in the aflatoxin biosynthetic pathway. Although not a direct part of the biosynthetic pathway, it modulates the expression of other key genes in aflatoxin production, helping to regulate biosynthesis as a whole. aflP. Function: Encodes an acyltransferase enzyme that is involved in the conversion of precursors into the final structure of aflatoxins (de Salud, 2002; Rojas Jaimes *et al.*, 2021). This enzyme facilitates the chemical modifications of precursors, allowing their transformation into functional aflatoxins. Aflatoxin biosynthesis is also regulated by several transcription factors and genes that modulate the overall expression of secondary metabolite biosynthesis in Aspergillus: LaeA´s function: LaeA is a global regulator that controls the production of secondary metabolites, including aflatoxins. This gene activates the expression of the genes responsible for the biosynthesis of aflatoxins, forming part of a global regulatory system. Importance: Regulates aflatoxin production in response to environmental factors, such as nutrient availability. VeA´s function: Similar to LaeA, VeA is also a master regulator of secondary metabolite biosynthesis. It is involved in the regulation of aflatoxin production by controlling the expression of genes such as aflR and others related to biosynthesis. Importance: VeA is activated under specific light and temperature conditions, which influences the production of aflatoxins. AflS´s Function: AflS acts as a negative regulator, inhibiting the production of aflatoxins by blocking AflR activity. Importance: The presence of AflS is crucial in the regulation of aflatoxin biosynthesis, as it prevents their production under unfavorable conditions, functioning as a negative modulator in the biosynthetic pathway (Perrone and Gallo, 2016; Setlem and Ramlal, 2022; Vásquez,

2025a; Vásquez, 2025b; Yu *et al.*, 2004; Yu *et al.*, 2007).

Based on the phylogenetic tree obtained from the three sequences analyzed, it is observed that Aspergillus_salvadorensis (UES-001, complete ITS) is separated from the other two sequences with a relatively high genetic distance. The calculated distances, expressed as *1 – identity*, range from 0.53 to 0.67, indicating a low level of direct similarity between the sequences compared. Within the group formed by Aspergillus flavus (chromosome 8) and Aspergillus parasiticus (cluster of genes of the aflatoxin pathway), a greater relative closeness between them is observed (distance ≈ 0.53) compared to *A. salvadorensis*, which has greater distances compared to both (≈ 0.63–0.67). This relationship is coherent from a biological point of view, since *A. flavus* and *A. parasiticus* are closely related within the genus *Aspergillus*, particularly within the *Flavi group*, associated with the production of aflatoxins. On the other hand, the sequence corresponding to *A. salvadorensis* appears as the most divergent in this analysis, forming a separate branch of the cluster that groups *A. flavus* and *A. parasiticus*. This suggests that isolation represents a genetically distinct entity with respect to the species compared in this preliminary study. It is important to note that the comparison was made between non-equivalent genomic regions: an ITS sequence (ribosomal region used for fungal identification) was compared against a complete chromosomal fragment and a specific gene cluster. This difference in the nature and length of the sequences can artificially increase the observed genetic distances and, therefore, the tree obtained should be interpreted as exploratory and not definitive.

The multiple alignment performed in MEGA 11 between *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus salvadorensis* shows a high level of similarity between the three sequences, evidenced by the large number of conserved positions where nucleotides coincide in all three species. This indicates that they share a close evolutionary relationship and a common ancestor, which is consistent with their belonging to the *Flavi* complex. Despite this general similarity, some specific differences are also observed at the nucleotide level, mainly in the form of single nucleotide polymorphisms (SNPs), which allow molecular distinction between each species. Visually, *A. flavus* and *A. parasiticus* show a greater coincidence with each other, while *A. salvadorensis* shows a slightly greater divergence, although it is still genetically close. The absence of large insertions or deletions in the analyzed fragment indicates that it is a conserved region and suitable for phylogenetic comparisons, which allows this information to be used for the construction of a reliable phylogenetic tree and for studies of identification and genetic diversity within the group.

CONCLUSION

The use of ultraviolet light to determine aflatoxins is suggestive of finding sufficient quantities present in the seeds, possibly used to identify contaminants to fungi. Some of the most relevant metabolites that could be common between *A. neoniger and A. salvadorensis* in terms of carcinogenic potential are aflatoxins. They are not the main ones, but they can be produced in low amounts if you produce them accordingly, as well as your genes present. Among the aflatoxin genes that could be involved as precursors of cancer are those of the aflR type. From the species to exposure to Ultraviolet light, there is a bright yellowish-green or faint to violet fluorescence, depending on the amount of toxin present in them. Of the most common species of the genus *Aspegillus* that produce aflatoxins, there are *A. flavus, A. pararasiticus, A. nomius, A. ochraceus, A. wentii and A. salvadorensis* under optimal temperature and humidity conditions. The *genes* aflA, aflB, aflC, aflD, aflE, aflG and aflJ, responsible for the conversion of precursors into aflatoxins such as AFB1 and AFG1, were not found in the salvadorensis species, therefore, it is concluded that it is not carcinogenic, because citing "The analysis results indicated that gene family UniRef90_M2YIY2: Dothistromin biosynthesis

regulatory protein aflR, UniRef90_O42716: Aflatoxin cluster transcriptional coactivator aflS genes related to aflatoxin were not detected. "., the text indicates that, in a genetic analysis, no sequences corresponding to two specific genes (identified by UniRef and their function), which are typically associated with the regulation of the production of aflatoxins and related compounds, were found. In summary it indicates the Regulatory Genes (aflR and aflS). The synthesis of these powerful toxins in the fungus is not random; it is controlled by a genetic "switch": aflR (Biosynthesis Regulatory Protein): This is the key regulatory gene. It acts as a transcription factor; that is, it is the protein that binds directly to the mushroom's DNA to "turn on" or activate the entire chain of genes necessary to produce aflatoxin. aflS (Transcriptional Coactivator): This gene, often called aflJ in other species, encodes a protein that works in conjunction with aflR. It acts as a co-activator, aiding and enhancing aflR's ability to activate aflatoxin genes. Bottom line: If these genes (aflR and aflS) are present and active, the fungus has the genetic potential to produce aflatoxins. The Mention of "Dothistromin", Dothistromin: It is a toxin produced by other fungi, such as *Dothistroma septosporum*, which attacks pine trees. Chemically, dotistromine is structurally similar to an aflatoxin precursor. Genetic Link: The aflR gene mentioned (UniRef90_M2YIY2) is identified here as a regulatory protein of dotistromine biosynthesis. This means that aflR in the organism tested is more closely related to the regulation of dotistromine. However, due to biochemical similarity, this gene is part of the family of aflatoxin-related toxin regulators. General conclusion of the result. "A search was conducted for the genetic 'switches' that allow fungi to produce the dangerous aflatoxin toxins (or very similar compounds), and none of those key genetic switches were found." Implication: The absence of these essential regulatory genes strongly suggests that the fungal organism tested does not possess the genetic capacity to produce aflatoxin. This is a positive conclusion in a risk analysis. Additional biochemical analyses are suggested to measure (quantify) possible very small amounts of secondary metabolites that are structurally similar to aflatoxins, even if the aflR and aflS regulatory genes are not found.

Bioinformatics programs used:

BLAST. https://blast.ncbi.nlm.nih.gov/Blast.cgi

CLUSTAL. https://www.ebi.ac.uk/jdispatcher/msa/clustalo?stype=protein

GENBANK.https://www-ncbi-nlm-nih-gov.translate.goog/genbank/?_x_tr_sl=en&_x_tr_tl=es&_x_tr_hl=es&_x_tr_pto=tc

UGENE https://ugene.net/

MEG11. https://www.megasoftware.net/

PHYTON. https://www.programiz.com/python-programming/online-compiler/

T-Cofee. https://tcoffee.crg.eu/apps/tcoffee/do:regular

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CONFLICT OF INTEREST

The author declares that he has no conflicts of interest.

REFERENCES

Adhikari, B.N., Bandyopadhyay, R., Cotty, P.J., 2016. Degeneration of aflatoxin gene clusters in *Aspergillus flavus* from Africa and North America. Amb Express, 6(1): 62.

Bolet Astoviza, M., Socarrás Suárez, M., 2005. Micotoxinas y cáncer. Rev. Cubana de Invest. Bioméd., 24(1): 54-59.

Caceres, I., Al Khoury, A., El Khoury, R., Lorber, S., P. Oswald, I., El Khoury, A., Atoui, A., Puel, O., Bailly, J.-D., 2020. Aflatoxin Biosynthesis and Genetic Regulation: A Review. Toxins., 12(3): 150.

Carvajal, M., 2013. Transformación de la aflatoxina B1 de alimentos, en el cancerígeno humano, aducto AFB1-ADN. TIP Revista Especializada en Ciencias Químico-Biológicas, 16(2): 109-120.

Cary, J.W., Ehrlich, K.C., Bland, J.M., Montalbano, B.G., 2006. The aflatoxin biosynthesis cluster gene, aflX, encodes an oxidoreductase involved in conversion of versicolorin A to demethylsterigmatocystin. Appl. Environ. Microbiol., 72(2): 1096-101.

Casquete, R., Benito, M.J., Aranda, E., Martín, A., Ruiz-Moyano, S., de Guía Córdoba, M., 2019. Gene expression of A*spergillus flavus* strains on a cheese model system to control aflatoxin production. J. Dairy Sci., 102(9): 7765-7772.

Chain, E.P.o.C.i.t.F., Schrenk, D., Bodin, L., Chipman, J.K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Leblanc, J.C., Nebbia, C.S., 2020. Risk assessment of ochratoxin A in food. EFSA J., 18(5): e06113.

Chalco Quezada, D.C., 2014. Riesgo toxicológico de aflatoxinas presentes en maní y nueces comercializados en los principales mercados de la ciudad de Cuenca.

de Salud, S., 2002. NORMA Oficial Mexicana NOM-188-SSA1-2002, Productos y Servicios. Control de aflatoxinas en cereales para consumo humano y animal. Especificaciones sanitarias. México DF: Diario Oficial de la Federación. Available at: http://www. salud. gob. mx/unidades/cdi/nom/188ssa12. html.

Faroni, L., Leda, R., 1993. Los granos y su calidad. Manual de manejo postcosecha de granos a nivel rural. Oficina Regional de la FAO para América Latina y el Caribe. Santiago, Chile: 250.

Illumina, 2025. Método de secuenciación Isep100.

Inc, M., 2025. Korea del Sur laboratoios.

Jallow, A., Xie, H., Tang, X., Qi, Z., Li, P., 2021. Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. Compr. Rev. Food Sci. Food Saf., 20(3): 2332-2381.

Kanehisa, M., Sato, Y., 2020. KEGG Mapper for inferring cellular functions from protein sequences. Protein Sci., 29(1): 28-35.

Keller, N.P., Hohn, T.M., 1997. Metabolic pathway gene clusters in filamentous fungi. Fungal Genetics and Biology, 21(1): 17-29.

Khaldi, N., Seifuddin, F.T., Turner, G., Haft, D., Nierman, W.C., Wolfe, K.H., Fedorova, N.D., 2010. SMURF: Genomic mapping of fungal secondary metabolite clusters. Fungal Genet. Biol., 47(9): 736-41.

Leiva Rivas, R.A., Guerrero Mendoza, Z.V., Nerio Vides, G.A., 2023. Determinación de la presencia de Aflatoxina B1 en masa de maíz (*Zea mays* L) de molinos artesanales del municipio de Suchitoto, departamento de Cuscatlán, El Salvador.

Mollay, C., Kimanya, M., Kassim, N., Stoltzfus, R., 2022. Main complementary food ingredients contributing to aflatoxin exposure to infants and young children in Kongwa, Tanzania. Food Contr., 135: 108709.

Perrone, G., Gallo, A., 2016. Aspergillus species and their associated mycotoxins. Mycotoxigenic fungi: Methods and protocols: 33-49.

Rojas Jaimes, J., Chacón Cruzado, M., Castañeda Peláez, L., Díaz Tello, A.,

2021. Cuantificación de aflatoxinas carcinogénicas en alimentos no procesados y su implicación para el consumo en Lima, Perú. Nutr. Hosp., 38(1): 146-151.

Setlem, S., Ramlal, S., 2022. Isolation, extraction and identification of aflatoxin producing *Aspergillus* fungi by HPLC analysis and ITS sequencing. Clin. Case Rep. Int., 2022; 6, 1393.

Vásquez, A., 2025a. Characterization of Aspergillus salvadorensis Isolated from *Caeselpinia coriaria* Seed, El Salvador.

Vásquez, A., 2025b. Phenotypic and Genotypic Characterization of *Aspergillus uessalvadorensis* in an Organic Strain Discovered at the University of El Salvador 2006-2024.

Yu, J., Chang, P.-K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk, C.P., Bennett, J.W., 2004. Clustered pathway genes in aflatoxin biosynthesis. Appl. Environ. Microbiol., 70(3): 1253-1262.

Yu, J., Ronning, C.M., Wilkinson, J.R., Campbell, B.C., Payne, G.A., Bhatnagar, D., Cleveland, T.E., Nierman, W.C., 2007. Gene profiling for studying the mechanism of aflatoxin biosynthesis in *Aspergillus flavus* and *A. parasiticus*. Food Addit. Contam., 24(10): 1035-1042.