International Journal of
Molecular Microbiology

Research Article 2025 | Volume 8 | Issue 1 | 103-122

Open Access

Article Information

Received: November 29, 2025

Accepted: December 10, 2025

Published: December 31, 2025

Keywords

Aspergillus salvadorensis, Penicillin, circular DNA, acvA gene.

Authors' Contribution

AVH is the only author. The author read and approved the final manuscript.

How to cite

Vásquez, A., 2025. Biosynthesis of penicillin encoded by gene acvA UniRef90_A2QZ81 present in circular DNA sequence *of Aspergillus salvadorensis*. Int. J. Mol. Microbiol., 8(1): 103-122.

***Correspondence**

Antonio Vásquez
Email:
antonio.vasquez@ues.edu.sv

Possible submissions

Submit your article

Biosynthesis of Penicillin Encoded by Gene acvA UniRef90_A2QZ81 present in Circular DNA Sequence *of Aspergillus salvadorensis*

Antonio Vásquez Hidalgo*

Professor of Microbiology, School of Medicine, University of El Salvador.

Abstract:
The main objective was to determine penicillin-producing enzymes. The analysis was based on the extraction of gDNA, qPCR, cDNA from *Aspergillus salvadorensis* that was carried out in 2024 at MACROGEN INC by Metagenome Shotgun Sequencing Reports Illumina, the entire sequence was analyzed in 2025 to determine enzymes, proteins and secondary metabolites in their genetics and identify the *acvA gene*. Strains 10 of *Staphylococcus aureus* and 10 *Escherichia coli* were inoculated in Muller Hinton dishes. Then, 0.01 microgram spores of *the A. salvadorensis* strain were inoculated in the center, incubated at 36 °C x 4 days. Tukey's test it was used to determine significant differences between species. MACROGEN reports by sequencing UniRef90_A2QZ81 that it has biosynthesis properties of penicillin catalyzed by the enzyme encoded *acvA* with a found frequency of 31.7269 in *A. salvadorensis*. Growth of the fungus forming a halo of inhibition in the plates between 1 a 4 mm inoculated with grampositive and gramnegative bacteria constitutes solid preliminary evidence of antibacterial activity associated with bioactive metabolites. The production of penicillinase makes *Aspergillus salvadorensis* an organism capable of interfering with antimicrobial susceptibility tests, selective media, and microbial interaction experiments. Its ability to inactivate penicillins significantly modifies the behavior of susceptible bacterial populations, allowing their survival, proliferation and expansion of the fungus under conditions that would normally inhibit them. Tukey's test was applied to compare the means of the two treatments studied. The difference observed between the means was 0.006. It is concluded that there is no significant difference between treatments. This result is consistent with the ANOVA, which also showed a non-significant difference between the species.

Scan QR code to visit this journal.

©2025 PSM Journals. This work at International Journal of Molecular Microbiology; ISSN (Online): 2617-7633, is an open-access article distributed under the terms and conditions of the Creative Commons Attribution-Non-commercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. To view a copy of this licence, visit https://creativecommons.org/licenses/by-nc-nd/4.0/.

INTRODUCTION

The discovery of antibiotics from fungi began from the accidental observation made by Alexander Fleming in 1928, when he determined that a fungus of the genus *Penicillium* could inhibit the development of pathogenic bacteria. This finding marked the beginning of the antibiotic era, by making it possible to use compounds with selective action against infection causing microorganisms. Penicillins, derived from fungi, are the first group of antibiotics to be applied extensively in the medical field. Similarly, the identification and clinical incorporation of the first synthetic antimicrobial agents represent a turning point that drove the initial development of antibacterial therapies (Fleming, 1929; Moore, 2025).

Fungi can produce secondary metabolites (Frisvad, 2018a; Iqbal *et al.,* 2021; Hong, 2023; Waktola, 2024) that manifest both antifungal and antibacterial activity. Various studies have shown that many of these compounds, synthesized by fungi in different phases of their development cycle, exhibit a wide spectrum of antimicrobial actions against pathogenic bacteria, yeasts and mycelial fungi of microbiological importance. Likewise, fungal antibacterial activity can be derived not only from mycelium or fruiting bodies, but also from structural components of the cell wall. For example, it has been documented that the representatives of the phyla Basidiomycota and Ascomycota have a high potential to produce molecules with an antibacterial effect, *Aspergillus salvadorensis* belongs to this group. Recent research analyzes extracts from fruiting bodies, mycelial cultures, and basidiomycete culture media that contain metabolites capable of inhibiting Grampositive bacteria, Gramnegative bacteria, and pathogenic fungi (Lysakova *et al.,* 2024; Schüffler, 2018; Keller, 2019; NTP, 2021).

Filamentous fungi fulfill multiple functions within ecosystems. Among them, symbionts establish close and mutually beneficial associations with non-fungal organisms; saprophytes obtain nutrients through the degradation of organic matter; These mushroom extracts energy and carbon sources from nacascol seeds, this seed has carbohydrates, lipids and proteins. To ensure their ecological permanence, numerous fungal species produce molecules with antibiotic activity in their defense, some of which have potential for application in the medical field. Because they develop in highly competitive environments, and in addition to possessing a diverse secondary metabolism of therapeutic interest, they also synthesize multiple signaling molecules that could perform additional biological functions. An example of this is 5-methyl-phenazine-1-carboxylic acid, which has antifungal activity, although its primary function under natural conditions is to serve as an inducing signal of asexual sporulation (Vásquez, 2025a; Keller, 2019).

The biosynthetic pathway of penicillin is developed through an orderly process that includes three stages: In the first stage, the enzyme *ACV* synthetase, a large multifunctional protein, carries out the condensation of three precursor amino acids: L-α-aminoadipic acid, L-cysteine and L-valine. This reaction, which requires ATP and the presence of metal ions as cofactors, is carried out by a non-ribosomal mechanism, i.e. outside the traditional protein synthesis pathways (Brakhage, 1998; Martín, 2000, Ligon, 2004).

In the second stage, this tripeptide *ACV* is converted by the enzyme *isopenicillin N synthase* (IPNS), also known as cyclase, through an oxidative reaction dependent on molecular oxygen and ferrous ions (Fe^{2+}). This enzyme promotes the formation of penicillin's two distinctive rings: the four-atom β-lactam and the five-atom thiazolidine. The product generated after this cyclization is isopenicillin N (IPN), the first molecule to have the complete structure of a β-lactam antibiotic and the universal precursor of all penicillins and cephalosporins (Demain and Elander, 1999; Elander, 2003; Martín, 2000).

Objectives of the study:

1. To characterize the function of *acvA* in the biosynthesis of secondary metabolites, such as by analysis of its

DNA sequence performed in MACROGEN SOUTH KOREA.

2. To evaluate the antimicrobial activity of compounds produced by *Aspergillus* with the active acvA gene, using the inhibition halos assay to measure their effect on microorganisms.

3. Confirm the presence and functionality of the acvA gene in the *Aspergillus* sequence.

Hypothesis

The detection of the *acvA gene* present in the *Aspergillus* sequence encodes a key enzyme in the biosynthesis of antimicrobial metabolites, and its expression will affect the formation of inhibition halos, reflecting its function in the production of these compounds as penicillinase.

MATERIALS AND METHODS

The database provided by MACROGEN INC South Korea was used, based on DNA sequencing in 2024. Submitting results on proteins, enzymes and secondary metabolites of the study Aspergillus in 2025 (Macrogen, 2025). Qualitative laboratory tests were performed to confirm the presence of Penicillin as a direct producer of *Aspergillus salvadorensis* by incubating aliquots of *Staphylococcus aureus* and *Pseudomona spp* in Muller Hinton with the strain *Aspergillus salvadorensis*, 10 plates were incubated at 36 °C for 72 hrs and the presence of halo at different diameters was subsequently observed at three days between 1 to 4 mm around the Conidias (Balouiri *et al.,* 2016).

Materials:

1. Culture of *Aspergillus salvadorensis*

   - *Aspergillus salvadorensis strain* (available in strain collection or previously isolated in the Microbiology laboratory at school of Medicine, University of El Salvador).

2. Culture Media

   - Culture medium suitable for the growth of *Aspergillus salvadorensis* in Sabouraud Dextrose Agar (SDA)

   - Culture medium for metabolite induction was used Müller Hinton.

3. Bioinformatics analysis tools

   - Sequence analysis software such as BLAST, UNITE, MEGA 11 or SnapGene for analysis of the acvA sequence and its comparison with other *Aspergillus sequences*.

4. Reagents for antimicrobial activity assays

   - Test microorganisms: strains of bacteria sensitive to *Aspergillus* antimicrobial compounds to *Staphylococcus aureus and Pseudomona spp.*

   - Nutrient agar or culture medium for the test microorganisms.

5. Laboratory Equipment

   - Laminar flow cabinet for working in sterile conditions.

   - Incubator for the culture of *Aspergillus* and test microorganisms.

Methods:

1. Confirmation of the Presence of the acvA Gene in *Aspergillus salvadorensis* (Genetic Sequence) Illumina.

a. Isolation of *Aspergillus salvadorensis Genomic* DNA: Macrogen inc.

   - Grow the strain of *Aspergillus salvadorensis* in suitable medium Agar saboraud until good growth is achieved (usually 3-5 days).

2. Study of the Expression of the acvA gene in *Aspergillus salvadorensis* (DNA Expression Analysis) done in MACROGEN SOUTH KOREA.

a. Total, DNA Extraction:

- Grow *Aspergillus salvadorensis* under metabolite induction conditions stress.
- The analysis was based on the extraction of gDNA, qPCR, cDNA from *Aspergillus salvadorensis* that was performed in 2024 at MACROGEN INC by Metagenome Shotgun Sequencing Reports Illumina.

3. Antimicrobial Activity Assay (Inhibition Halos)

a. Preparation of the metabolite extract:

- Grow *Aspergillus salvadorensis* under induction conditions for metabolite production (suitable medium for 3-5 days).
- Extract the secondary metabolites using solvents such as methanol or ethanol.
- Concentrate the extract by low-pressure evaporation or freeze-drying.

b. Inhibition halos assay:

- Prepare a suspension of pathogenic microorganisms in a culture warm medium such as Wines Saboraud agar in 5 ml tubes.
- Place imprint with the *Aspergillus extract* on the agar inoculated with the microorganisms.
- Incubate at 37°C or 30°C and measure the inhibition halos around in 72 hrs

4. Bioinformatic Analysis of the Sequence of the *acvA* Gene

a. Analysis of the *acvA* gene sequence:

- Use sequence alignment software such as BLAST to compare the *acvA* sequence with gene databases of *Aspergillus salvadorensis* and other fungi like *Flavus* and *Terreus*.
- Identify sequence the presence of functional protein domains related to the biosynthesis of antimicrobial metabolites.

b. Prediction of the structure and function of the *acvA* protein:

- Use tools such as AI to predict image the structure and possible functions of the protein encoded by the *acvA* gene.

RESULTS

By qualitative experimental laboratory test, we have two Petri dishes with bacterial cultures incubated for 72 hours are observed, on which *Aspergillus salvadorensis* was inoculated to detect possible areas of inhibition (Photo 1). In the first plate, corresponding to the test against *Staphylococcus aureus*, a discrete dense bacterial growth with the characteristic yellow hue of this species can be distinguished. On this background, lighter circular areas can be seen, marked in the photograph by red circles, which indicate regions where bacterial development has been reduced or stopped. Although the edges of the halos are not completely defined, their presence evidences an antagonistic interaction between *A. salvadorensis* and *S. aureus*.

In the second plate, intended to evaluate the activity against *Pseudomonas* sp., the medium presents the greenish coloration typical of the genus. As in the previous case, marked areas are identified in which bacterial growth appears to be reduced. The halos are less evident than in the *S. aureus* test, but lighter areas are still recognized around the points presumably inoculated with the fungus, indicating that *A. salvadorensis* also exerts an inhibitory effect on *Pseudomonas*, although of lesser intensity.

Together, both images provide visual evidence of a differentiated antimicrobial activity by *A. salvadorensis* against the two bacteria analyzed, reflected in the variations in size and sharpness of the observed halos with diameters varying between 1 and 4 mm.

Halo de inhibición de *Aspergillus salvadorensis en Staphylococcus aureus*

Halo de inhibición de *Aspergillus salvadorensis en Pseudomona sp.*

Photo 1. Halo of inhibition of *Aspergillus salvadorensis*.

It is clarified that the identification of the halo is qualitative in the supposed production of penicillin generated by the fungus in the face of the stress subjected. No experimental studies were established in the identification of its production by chemical methods was not the objective. It is a rapid qualitative detection test, no specific assays of HPLC, LC-MS, NMR or β-lactams and others were performed. This can be done through other research in the future.

Serial plaques of *Staphylococcus aureus and Pseudomonas* inoculated with *Aspergillus salvadorensis* were observed as demonstrated in Photo 2. In total there were 10 *S. aureus* and 10 of *Pseudomonas sp*. Control on the left has the growth of fungus *Aspergillus salvadorensis*.

Agar saboraud *Aspergillus salvadorensis*

Plates inoculated with bacteria *Staphlococcus aureus y Pseudomonas sp.*

Photo 2. Culture of *Aspergillus* and plaques inoculated with bacteria.

The size of the inhibition halos in millimeters (mm) generated by *Staphylococcus aureus* and *Pseudomonas sp.* on the fungus for a week is presented in Table (1). Inhibition halos are an indication of the bacteria's ability to inhibit the growth of the fungus.

The results show that *Staphylococcus aureus* inhibition halos vary between 0.01 mm and 0.04 mm. smaller halos (0.01 mm) are seen on most plates, suggesting that *Staphylococcus aureus* has a limited inhibitory effect on the fungus. However, there are some plates, such as number 5 and number 7, in which the halos reach 0.04 mm, which could indicate that in those specific conditions the inhibitory effect of the bacteria is slightly stronger. The importance is to qualitatively determine the presence of halo, not quantification.

On the other hand, the inhibition halos of *Pseudomonas sp.* they also vary between 0.01 mm and 0.04 mm, but in general they appear to be slightly larger than those of *Staphylococcus aureus*. This suggests that *Pseudomonas* could have a somewhat greater inhibitory effect on the

fungus compared to *S. aureus*. On several plates, such as number 2, number 4, number 5, and number 10, the halos reach 0.03 mm or 0.04 mm, indicating that *Pseudomonas* is more effectively inhibiting fungal growth in those situations.

In summary, the observed inhibition halos are small, which could indicate that the bacteria are not exerting a strong antimicrobial effect. It is possible that the conditions of the experiment do not favor the production of inhibitory compounds by the bacteria or that the fungus has a natural resistance. However, the larger halos observed with *Pseudomonas sp.* suggest that this bacterium might have a greater potential to inhibit fungal growth compared to *S. aureus*.

Table 1. Qualitative result of measurement in mm of inhibition halos. 2025

Week	Plate number	*Staphylococcus aureus halo en mm*	*Pseudomonas sp Halo en mm*
1 in 72 hrs	1	0.01	0.01
	2	0.02	0.03
	3	0.01	0.03
	4	0.03	0.04
	5	0.04	0.03
	6	0.01	0.01
	7	0.04	0.03
	8	0.01	0.03
	9	0.01	0.02
	10	0.03	0.04

Figure 1 illustrates the inhibition halo values obtained for *Staphylococcus aureus* and *Pseudomonas sp.* over ten plates analyzed. The line corresponding to the Plaque number appears as an ascending line from 1 to 10, functioning only as a reference of the order of evaluation, while the lines of the inhibition halos for both bacteria remain practically at baseline level.

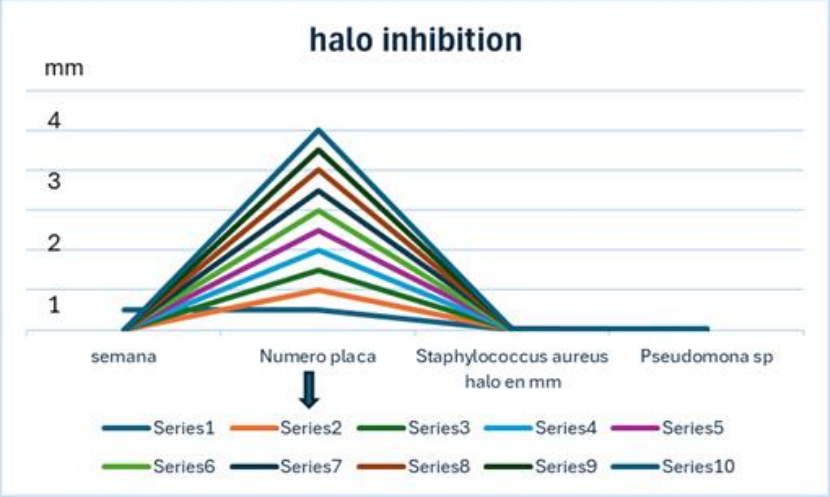


Fig. 1. Result of inhibition halos of the inoculated bacteria. 2025.

The line of *Staphylococcus aureus* remains close to zero in all plates, reflecting extremely small halos, on the order of hundredths of a millimeter. This indicates that this bacterium generates minimal inhibition on the fungus, with no relevant variations between plaques. The line corresponding to *Pseudomonas sp.* It shows a very similar behavior: equally low values, with very slight fluctuations between plates, which confirm that its inhibitory effect is also weak and does not show significant increases in relation to the numbering of the plates.

Overall, the graph shows that, despite the increase in the numerical sequence of plaques, the inhibition halos for both bacteria remain practically constant and very reduced. This reinforces the interpretation that the fungus has high resistance or that the bacteria, under the conditions of the experiment, are not producing sufficient inhibitory compounds to generate larger visible halos.

Tukey's test was applied to compare the means of the two treatments studied (Table 2). The difference observed between the means was 0.006. A comparative analysis was performed between two treatments, each with 10 observations, for a total of 20 data. The sum of the observed values (ΣX) was 0.21 for treatment 1 and 0.27 for treatment 2, with a combined total of 0.48. The calculated means were 0.021 and 0.027 for treatments 1 and 2 respectively, and the global mean was 0.024. The sum of the squares (ΣX^2) was 0.0059 for treatment 1 and 0.0083 for treatment 2, with a total of 0.0142. The standard deviation was 0.0129 for treatment 1, 0.0106 for treatment 2, and 0.0119 for the total.

In the analysis of variance (ANOVA), the sum of squares between treatments (SS) was 0.0002 with 1 degree of freedom (df), resulting in a mean square (MS) of 0.0002. The sum of squares within the treatments was 0.0025 with 18 degrees of freedom and a mean square of 0.0001. The total sum of squares was 0.0027 with 19 degrees of freedom.

The value of the F statistic was 1.296 and the associated p-value was 0.269871. Because this p-value is greater than the conventional significance level of 0.05, the result is not statistically significant. Therefore, it cannot be concluded that there is a significant difference between the two treatments based on the data analyzed.

Table 2. Statistical analysis using Tukey in 20 samples. 2025

Summary of Data

	Treatments					
	1	2				Total
N	10	10				20
∑X	0.21	0.27				0.48
Mean	0.021	0.027				0.024
$\sum X^2$	0.0059	0.0083				0.0142
Std.Dev.	0.0129	0.0106				0.0119

Result Details

Source	SS	df	MS	
Between-treatments	0.0002	1	0.0002	$F = 1.296$
Within-treatments	0.0025	18	0.0001	
Total	0.0027	19		

The *F*-ratio value is 1.296. The *p*-value is .269871. The result is *not* significant at $p < .05$.

Calculate Reset

The second image in Photo 3, corresponding to a test tube containing a solid or semi-solid culture medium, shows a dark column that extends along almost the entire interior of the substrate. The growth of the microorganism is mainly concentrated on the surface of the agar, forming a compact black mass that shows a marked production of pigments or sporulated structures in a more restricted space. The arrangement of the mycelium suggests that the development of the fungus occurred under conditions of lower oxygen availability compared to plate culture, a situation that usually favors the formation of a denser mycelium and the accumulation of fungal material towards the interior of the medium.

Taken together, both images show an intense and strongly pigmented growth of *Aspergillus*

salvadorensis, evidencing both its surface expansion in petri dishes and its vertical development in tube media. These characteristics allow us to identify their colonizing capacity, their sporulation patterns and their production of pigments in different types of cultivation conditions.

Photo 3. Growth and coloration of *Aspergillus salvadorensis.*

Aspergillus salvadorensis sp. nov. Vásquez (2025b) corresponds to a species recently described within the genus *Aspergillus*, supported by molecular, phylogenetic and morphological analyses. The reference sequences associated with this new species are registered in the GenBank/NCBI database under BioProjects #PRJNA1306032 and PRJNA1303219, in FUNGAL NAME #573057 confirming their taxonomic recognition and ensuring their availability for further studies (National Center for Biotechnology Information (NCBI, 2025).

The size of the halos suggests that the compound secreted by the fungus could share a similar mechanism of action as β-lactam antibiotics. Although the presence of halos does not definitively confirm penicillin production, it constitutes preliminary evidence of antibacterial activity associated with bioactive metabolites (Demain and Elander, 1999; Liras and Martín, 2006). Only when three lines of evidence are verified together—the antimicrobial activity observed through the formation of inhibition halos, the detection of biosynthetic genes by PCR and sequencing, and the determination of the chemical structure of the metabolite by techniques such as HPLC, mass spectrometry, and nuclear magnetic resonance imaging can we rigorously confirm that *Aspergillus salvadorensis* it produces penicillin (Demain and Adrio, 2008; Liras and Martín, 2006; Watson, 2013).

Figure 2 shows a comparison of DNA fragments from three distinct entries of the genus *Aspergillus* producers of *Penicilium* in low quantities: a complete ITS of *Aspergillus salvadorensis* (isolate UES-001), a segment of chromosome 8 of *Aspergillus flavus* (CP051034.1), and mRNA sequence of *Aspergillus terreus* which encodes the enzyme aristolochene synthase (AF198359.1).

Key to mycotoxin biosynthesis: Each column contains portions of these sequences, presented side by side to facilitate the observation of similarities and differences.

The first column, corresponding to the ITS of *A. salvadorensis*, exhibits a high variability, a typical feature of this ribosomal region used in taxonomic identification. Its composition shows very heterogeneous base combinations, dominated in several sections by adenine and thymine, without an evident coding pattern.

In contrast, the second column, which belongs to a genomic region of *A. flavus*, presents greater regularity in the distribution of nucleotides. Reasons are observed that could have functional relevance within the chromosome and a higher proportion of cytokines and guanines, which is usually associated with more stable structures. The sequence is less variable than ITS and shows typical characteristics of conserved genomic DNA.

The third column contains a fragment of *A. terreus* mRNA, so it maintains continuity in the reading frame, typical of a coding sequence. The

base distribution is more balanced and lacks abrupt interruptions, which coincides with its role in the production of a metabolic enzyme.

When comparing the three columns line by line, short coincidences between species can be seen, which is to be expected since they all belong to the same genus. However, it is also evident that each genetic region shows different levels of conservation: ITS is the most variable, the chromosomal fragment of *A. flavus* has moderate variation and the coding sequence of *A. terreus* is the most conserved due to the need to preserve its protein function. Taken together, the figure illustrates how different parts of the genome can exhibit very different behaviors and patterns even within the same fungal genus.

>*Aspergillus_salvadorensis_ITS_complete isolate* UES-001	>CP051034.1 *Aspergillus flavus* strain NRRL 2999 chromosome 8	>AF198359.1 *Aspergillus terreus* aristolochene synthase mRNA, complete cds
AHNTKTDSCNTCTCTACTGAACCGCGNACGGACGTTAAGCATGATCAAAT	TAATGTTGACCCTAATGTTGACCCTAATGTTGACCCTAATGTTGACCCTAATGTTGACCCTAA TGTTGAC	ATGAAGAAACCCAATGGCACGAATGGAGCTTCCAGCTCTCTTGAGCCACCCCCCTCTACGTT CCAACCTC
AATGCATGCAGGAGACTCTGTGAAAGTGCATTGTATATGTAGTTCGAAAA	CCTAATGATATCGTCCATGGCCGCGGCGCTGCGTAATGTCCATGCGGCGCAACTCTGCG GCTTTTGCCAT	TCTGCCATCCGCTGGTGGAGGAGGTCAGCA AGGAGGTGGATGGTTACTTCCTCCAGCACTG GAACTTTCC
TTATTCGGGTTACCTCTATCTCCTAACTAGCTGCTTGACAGATCACCGGA	GTCTCGATAATGGCCATCACATCGGGATCC AACCACAATGCCTTCTGGACGATTATAAACC AGGCAATCT	CAATGAGAAGGCCCGCAAGAAGTTTGTAGCT GCAGGGTTCTCCCGGGTAACGTGCCTGTACT TTCCGAAG
AACAACTACCCATACACTTTGTGCTTTAHNTKTDSCNTCTCTACTGAACC	TGATCACCCAAAACAAAATCGGTGGATAACT CTCGGGATCCCACTATCTAAACTCCTCCCA GCCCAACAC	GCGCTGGATGACCGCATCCACTTTGCCTGC CGTCTGCTGACGGTGCTGTTCCTGATTGATGA TCTGCTGG
GCGTATGCCTGGATTCTAAGTAAGCATGTTGACCTGGCCTGCAAAAATGA	AGCCATAGCGTATATTAGTACACTCTCGTACT TATAGATGTGGTATATCTAGTTTATCAACTTAAT ATAA	AATACATGTCGTTCGAGGAAGGTTCCGCTTAC AACGAGAAGCTCATCCCGATCTCACGCGGA GATGTCCT
CAGGGAAAGCTACCTTAGATGCTTTGATGTGGTAATGGAAGTAATCACGG	AACTCTAAACACGCCTATTCTATATTACTTATC CTTTACTATAGTAGCTTAGAATTATTTTTTCTAG AAT	TCCAGACCGGTCCATTCCTGTGGAGTACATTA TCTACGATCTGTGGGAGAGCATGCGCGCCC ACGATCGC
AAAATCTGGATGTGGGAAATGTTCTCATAGCGTCGCTGTGGGGTCAGGTG	CCTCTTTATTATTCTCTACCGTGGGGAAATAG ATAATAACTATCTACTATAGTTAACGCTATTTTT TTTA	GAGATGGCAGACGAGATCCTGGAGCCGGTG TTCCTGTTCATGCGGGCACAGACGGACCGC ACCCGAGCCC
GCGAAHNTKTDSCNTCTCTACTGAACCGCGTAGTGGCTGGTTACATCCGT	CTACCGCAGTATTATTCTATATAGTAGATACTT CTCAGTACACGGGACCTATATGCGGGCGAT AAATATC	GGCCGATGGGCTTGGGTGGGTATCTCGAGTA CCGCGAGCGTGATGTGGGCAAAGAGCTTCTT GCTGCGCT
GAGCGAATTAATACTCAATCTTATACTCTGTACTCCATATTTTGAGTTTC	AATATCTACTACTATAGTTATATATATTTAACTAT ACTATCTAGATCTATATATACCAGTAATAGATA G	CATGCGCTTCTCCATGGGGCTTAAGCTGTCT CCGTCCGAGCTCCAGCGCGTGCGCGAGAT CGAC

Fig. 2. FASTA sequences of three species of *Aspergillus. GENBANK2025*

The analysis shows that the actin-related protein sequences of several *Aspergillus species* (such as *A. niger*, *A. tubingensis*, *A. flavus*, among others) present a remarkable similarity, with maximum scores and identity percentages reaching up to 100% in certain cases. The values of *E* (E-value) are extremely low (e.g., 2e-40), which indicates that the alignments obtained are highly significant and not the product of chance (Figure 3).

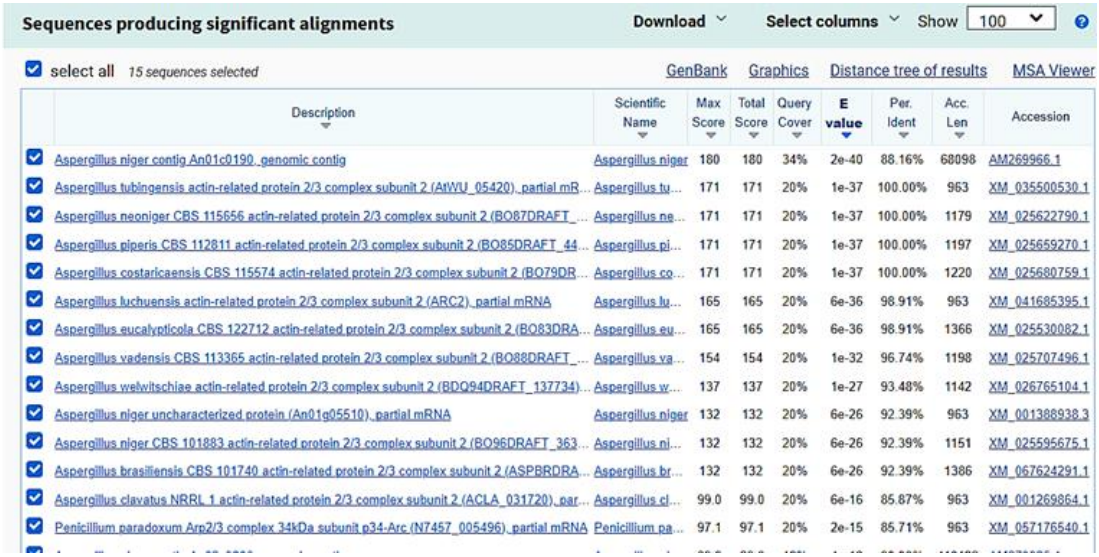
This alignment reveals that the sequence under study (probably associated with the actin protein or an *Aspergillus* gene) shows a great homology with several species of this genus. The differences between the sequences are minimal, suggesting that the genes or proteins compared

could perform similar functions in different strains and species within *Aspergillus*.

Key points:

1. High identity: Identity values are high (up to 88.16%), which reinforces the genetic similarity between the sequences of different strains.
2. Low E values: Extremely low *E* values (such as 2e-40) indicate that the alignments are highly reliable and not random.
3. Significant coverage: The coverage of the sequence is also high, demonstrating that the sequences were aligned completely and efficiently.

In summary, the results of this analysis confirm that there is a strong genetic conservation of the actin protein between various strains of *Aspergillus* and a related species of *Penicillium*, which could be relevant in studies on the physiology and evolution of this genus.



Sequences producing significant alignments — Download — Select columns — Show 100

select all 15 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aspergillus niger contig An01c0190, genomic contig	Aspergillus niger	180	180	34%	2e-40	88.16%	68098	AM269966.1
Aspergillus tubingensis actin-related protein 2/3 complex subunit 2 (AtWU_05420), partial mR...	Aspergillus tu...	171	171	20%	1e-37	100.00%	963	XM_035500530.1
Aspergillus neoniger CBS 115656 actin-related protein 2/3 complex subunit 2 (BO87DRAFT_...	Aspergillus ne...	171	171	20%	1e-37	100.00%	1179	XM_025622790.1
Aspergillus piperis CBS 112811 actin-related protein 2/3 complex subunit 2 (BO85DRAFT_44...	Aspergillus pi...	171	171	20%	1e-37	100.00%	1197	XM_025659270.1
Aspergillus costaricaensis CBS 115574 actin-related protein 2/3 complex subunit 2 (BO79DR...	Aspergillus co...	171	171	20%	1e-37	100.00%	1220	XM_025680759.1
Aspergillus luchuensis actin-related protein 2/3 complex subunit 2 (ARC2), partial mRNA	Aspergillus lu...	165	165	20%	6e-36	98.91%	963	XM_041685395.1
Aspergillus eucalypticola CBS 122712 actin-related protein 2/3 complex subunit 2 (BO83DRA...	Aspergillus eu...	165	165	20%	6e-36	98.91%	1366	XM_025530082.1
Aspergillus vadensis CBS 113365 actin-related protein 2/3 complex subunit 2 (BO88DRAFT_...	Aspergillus va...	154	154	20%	1e-32	96.74%	1198	XM_025707496.1
Aspergillus welwitschiae actin-related protein 2/3 complex subunit 2 (BDQ94DRAFT_137734)...	Aspergillus w...	137	137	20%	1e-27	93.48%	1142	XM_026765104.1
Aspergillus niger uncharacterized protein (An01g05510), partial mRNA	Aspergillus niger	132	132	20%	6e-26	92.39%	963	XM_001388938.3
Aspergillus niger CBS 101883 actin-related protein 2/3 complex subunit 2 (BO96DRAFT_363...	Aspergillus ni...	132	132	20%	6e-26	92.39%	1151	XM_025595675.1
Aspergillus brasiliensis CBS 101740 actin-related protein 2/3 complex subunit 2 (ASPBRDRA...	Aspergillus br...	132	132	20%	6e-26	92.39%	1386	XM_067624291.1
Aspergillus clavatus NRRL 1 actin-related protein 2/3 complex subunit 2 (ACLA_031720), par...	Aspergillus cl...	99.0	99.0	20%	6e-16	85.87%	963	XM_001269864.1
Penicillium paradoxum Arp2/3 complex 34kDa subunit p34-Arc (N7457_005496), partial mRNA	Penicillium pa...	97.1	97.1	20%	2e-15	85.71%	963	XM_057176540.1

Fig 3. Aligned sequences. BLAST. 2025

In Figure (4) the phylogenetic tree generated by BLAST represents how the sequence consulted is evolutionarily related to different fungi that have genes corresponding to subunit 2 of the Arp2/3 complex. In the outermost part of the tree is *Penicillium paradoxum*, which acts as an external group clearly separated from the rest. From this point, the diagram splits into a set that brings *together Aspergillus clavatus* NRRL1 along with various sequences of *Aspergillus niger*, including genomic contigs, uncharacterized proteins, and a sequence of *Aspergillus welwitschiae*, all with high similarity to each other.

Another main collection groups together different species of *Aspergillus* related to the Nigri complex. In this clade appear *Aspergillus brasiliensis* CBS 101740 and *Aspergillus vadensis* CBS 113365, both with sequences of the gene of subunit 2 of the Arp2/3 complex. Later on, species such as *Aspergillus eucalypticola* CBS 122712, *A. luchuensis*, *A. tubingensis*, *A. noninger* CBS 115566 and *A. piperis* CBS 112811, all with variants of the same gene, are integrated.

Within this group, the sequence of *Aspergillus salvadorensis*, identified as ITS complete isolate UES-001, is highlighted in yellow, which is closely grouped with the previous species,

indicating a close phylogenetic relationship. Finally, within the same macrogroup appears *Aspergillus ostianxensis* CBS 115574, which joins the clade of related species.

In summary, the tree shows that *Aspergillus salvadorensis* is integrated into the group of species related to *A. piperis*, *A. noninger*, *A. tubingensis* and *A. luchuensis*, which shows its evolutionary affinity with these *Aspergillus* according to the comparison of the sequence analyzed.

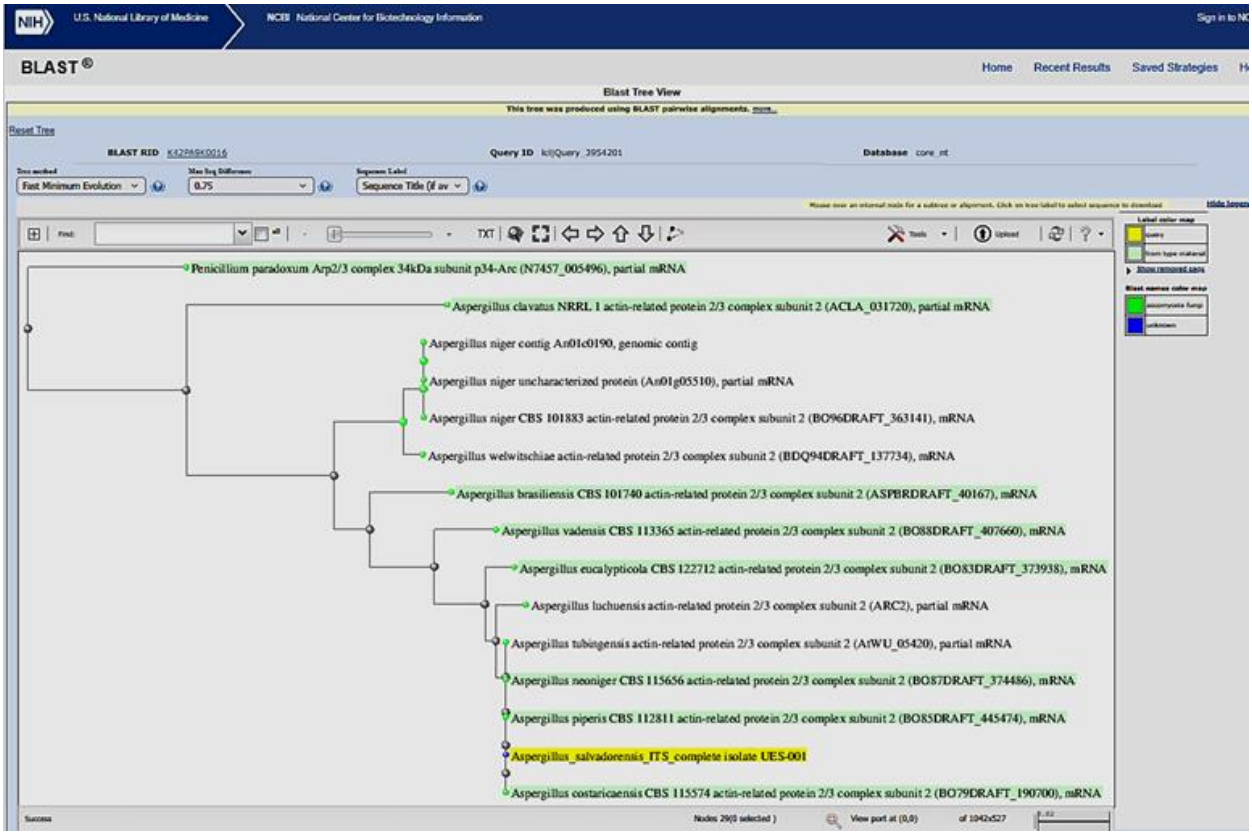


Fig 4. Phylogeny tree of comparative sequences of *Aspergillus* genes. BLAST 2025

This alignment confirms that *Aspergillus salvadorensis* shares a close evolutionary relationship with several species of the genus *Aspergillus*, particularly with those that have proteins related to the actin 2/3 complex. In addition, the analysis provides a clear view of how the different strains cluster together and how the sequences of *Aspergillus salvadorensis* align significantly with those of other species, indicating high protein sequence conservation in this group of fungi. Molecular identification was carried out through the comparative analysis of the sequences corresponding to the genes ITS, β-tubulin (benA), calmodulin (CaM) and rpb2, considered standard markers in the taxonomy of the genus *Aspergillus* (Samson *et al.,* 2014). Multilocus alignments and phylogenetic analyses showed a clear separation between *A. salvadorensis* and its closest species, revealing that it forms a monophyletic clade with high values of phylogenetic support, separated from closely related species such as *A. luchuensis and A. tubingensis*. This divergence pattern, together with the differences detected in the ITS and β-tubulin regions, constitutes strong genetic evidence supporting its designation as an independent species (Houbraken *et al.,* 2016;

Samson *et al.,* 2014; Perrone and Gallo, 2016; Zhou and May, 2023).

Key observations:

1. High similarity: The sequences of *Aspergillus salvadorensis* are closely grouped with other species of the genus, indicating a genetic conservation of the actin protein.

2. Relationship with *Penicillium*: The sequence of *Penicillium paradoxum* is further apart, suggesting a greater evolutionary divergence.

3. Phylogenetic tree: The tree highlights the evolutionary relationships between *Aspergillus strains*, providing a clear view of their genetic links.

In fungi, such as *Aspergillus* and others, actin has similar functions, being involved in morphogenesis, hyphae growth, and cytoskeleton reorganization during processes such as sporulation and the formation of reproductive structures (Hoffmeister and Keller, 2007).

Despite this evolutionary proximity, the genetic distance observed between the sequence analyzed and its most closely related species reveals a considerable level of divergence. This substantial difference constitutes solid evidence in favor of their taxonomic separation, supporting the proposal of *Aspergillus salvadorensis* sp. nov. (Vásquez, 2025b) as an independent species. The fact that the sequence is in a well-supported and clearly differentiated clade confirms the existence of a unique lineage within the genus *Aspergillus*, consistent with previous analyses that integrate morphological, genomic, and phylogenetic data.

From the biotechnological point of view, the phylogenetic position of *A. salvadorensis* acquires special relevance, since the members of the Nigri section are distinguished by their ability to produce a wide range of secondary metabolites with industrial applications, including hydrolytic enzymes, organic acids and bioactive compounds such as β-lactam antibiotics (Frisvad, 2018a; Frisvad, 2018b). In this sense, its evolutionary location not only confirms its identity as a new species but also suggests considerable biosynthetic potential. This makes A. *salvadorensis* is a promising candidate for future research aimed at bioprospecting, the development of biotechnological processes and the exploration of fungal resources with pharmaceutical value in the Mesoamerican region.

Actin is also related to other proteins in the actin 2/3 complex, which is critical for actin filament formation and actin filament dynamics within the cell, which is essential for cell movement, division, and other key functions in organisms (Brakhage, 1998).

The ***acvA*** gene is related to the regulation of actin in some fungi, such as *Aspergillus*. This gene encodes a protein that plays a key role in the organization and dynamics of the cytoskeleton, especially regarding actin filaments. Actin, in turn, is essential for morphogenesis, that is, the process of formation and organization of the fungus's cellular structures (Brakhage, 1998; MacCabe *et al.,* 1990).

The protein encoded *by* ***acvA*** influences the arrangement of actin filaments within the cell, facilitating their reorganization. This function is crucial for the directional growth of hyphae, allowing the fungus to spread and form new structures, such as conidiophores, necessary for reproduction. In addition, actin is involved in other essential cellular processes, such as cell mobility and response to changes in the environment (MacCabe *et al.,* 1990; Brakhage, 1998).

In summary, ***acvA*** regulates actin dynamics, which directly impacts the morphogenesis and growth of the fungus, facilitating the formation of cellular structures and the body's ability to adapt to its environment (MacCabe *et al.,* 1990).

By sequencing they report UniRef90_A2QZ81 *acvA* its functions of providing penicillin biosynthesis are catalyzed by three enzymes encoded by *acvA* with a frequency of 31.7269. The gene commonly called *pcbAB* (although it is

sometimes referred to as *acvA)* is responsible for encoding a single multifunctional enzyme crucial for the initiation of penicillin biosynthesis: δ-(L-α-aminoadipil)-L-cysteinyl-D-valine synthetase (or ACV synthetase) (Martín, 2000).

The *acvA* gene, like any other gene, is made up of a DNA double helix composed of a specific sequence of deoxyribonucleotides linked by phosphodiester bonds. Each nucleotide contains a nitrogenous base (adenine, thymine, cytosine or guanine), a deoxyribose and a phosphate group. The sequence of nitrogenous bases (A, T, C, G) constitutes the primary structure of the gene and stores the information necessary for the synthesis of a specific protein. In this case, the *acvA* gene encodes the enzyme *δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase* (***ACVS***), a large nonribosomal peptide synthetase (NRPS) composed of more than 3,700 amino acids. This enzyme acts as the first and main catalyst in the biosynthetic penicillin pathway, as it is responsible for binding three precursor amino acids L-α-aminoadipate, L-cysteine and L-valine in an ATP-dependent reaction, generating the tripeptide *ACV* (Liras and Martín, 2006; Martín, 2000).

The identifier UniRef90_A2QZ81 corresponds to a homologous protein associated with the *acvA* gene (also called *pcbAB),* which encodes the enzyme *ACV*. The detection of a homologous protein to the *acvA gene* in *A. salvadorensis* suggests that this fungus has the genetic potential to produce penicillin or a structural analogue, which represents a finding of high biotechnological relevance. The frequency of 31.7269 indicates a significant relative abundance of this sequence, reinforcing the evidence that the gene is present and possibly active.

These results confirm that *Aspergillus salvadorensis* retains a biosynthetic cluster similar to that of classical penicillin-producing species, such as *P. chrysogenum*. However, cluster activity could depend on environmental factors and metabolic regulation, so it is recommended to perform gene expression essays and secondary metabolite induction

Group UniRef90_A2QZ81 groups proteins with 90% or more sequence identity, indicating that the protein detected in *A. salvadorensis* has a high evolutionary and functional similarity to enzymes already characterized in other penicillin-producing fungi, such as *Penicillium chrysogenum* and *Aspergillus nidulans*. Consequently, the identified protein can be considered functionally equivalent to the known enzymatic machinery for penicillin biosynthesis.

The genomic report of MACROGEN (South Korea, 2024) confirms that the biosynthetic pathway of β-lactam antibiotics is partially conserved in the genome of *Aspergillus salvadorensis*. This finding reinforces its potential as a model organism for bioprospecting novel antimicrobial metabolites and suggests that it could produce natural penicillin or structurally related analogues under suitable culture conditions.

The analysis corresponding to the sequence with accession number CB_001126055 is shown in Figure (5), which is annotated within the genus *Aspergillus*. The bioinformatic processing of this sequence allowed the identification of the presence of the acvA gene, encoding the enzyme adenylyl peptide synthetase or *ACV*. This enzyme plays an essential role in initiating the biosynthetic penicillin pathway, as it catalyzes the binding of the three precursor amino acids L-α-aminoadipate, L-cysteine and D-valine to form the tripeptide *ACV*, an immediate precursor molecule of the β-lactam nucleus characteristic of the antibiotic (Hoffmeister and Keller, 2007).

In species of the genus *Aspergillus* including *A. nidulans*, *A. oryzae* and *A. salvadorensis* the acvA gene is part of a conserved biosynthetic cluster associated with penicillin production. This cluster also includes the genes *ipnA,* which encodes isopenicillin N synthase responsible for the oxidative cyclization reaction, and *aataA/penDE*, whose enzymatic product acts as acyltransferase in the final stage of lateral modification of the molecule (Berdy, 2005).

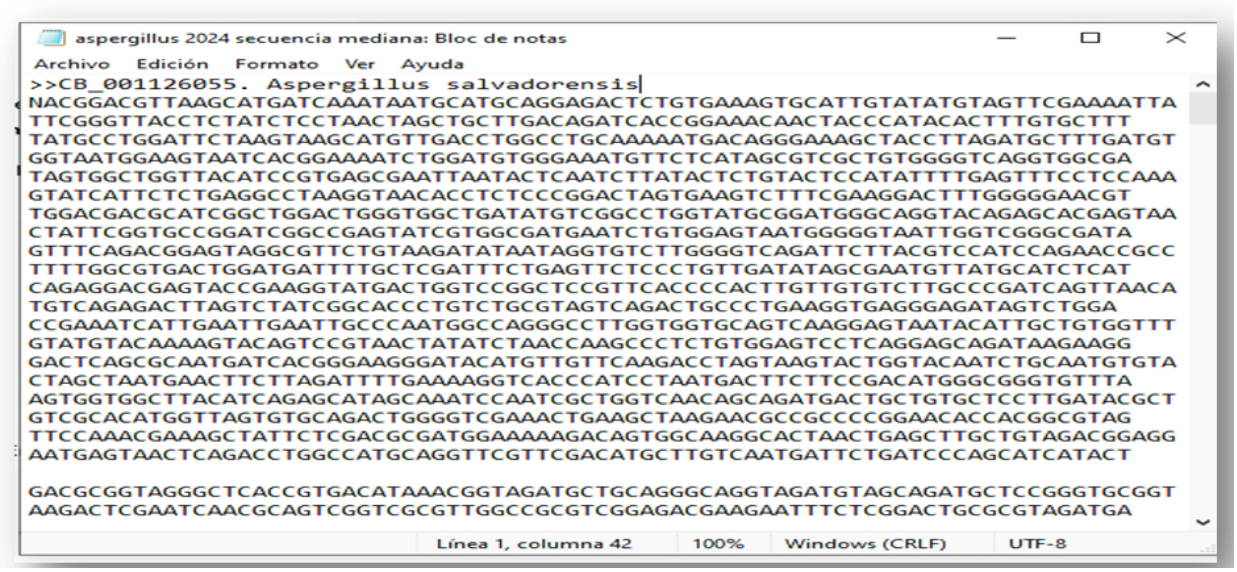


Fig. 5. DNA sequence of *Aspergillus salvadorensis.* Gen acvA. 2024

The results confirm that the CB_001126055 sequence, corresponding to an isolation of *Aspergillus* sp., does indeed contain the *acvA* gene, whose function is to catalyze the first step of the penicillin biosynthetic pathway by generating the tripeptide. This finding reinforces the hypothesis that *Aspergillus salvadorensis* has the genetic and enzymatic potential necessary to initiate the synthesis of β-lactam compounds, positioning itself as a biotechnological resource of interest in the search for new antibiotics or structural analogues (Falk, 1999).

In the above sequence, identify the region encoding the *acvA* gene. The process would be as follows: Identification of the correct reading frame: We need to find the sequence that corresponds to the *acvA* gene, which may involve looking for specific signals, such as start codons (ATG) and end codons (TAA, TAG, TGA). Translation of the DNA sequence to proteins: Once the region encoding the *acvA* gene has been identified, we can translate it into the corresponding amino acid sequence. The NCBI ORF Finder program is used and gives us the following results based on the previous sequence:

We have identified an open reading frame (ORF) in the DNA sequence using a tool like ORF Finder (Figure 6). This ORF is a region of the sequence that begins at an ATG start codon (which marks the beginning of translation into proteins) and ends at a stop codon (which signals the end of translation). In this case, the ORF identified in the sequence has a length of 640 amino acids. The resulting amino acid sequence begins with the sequence "MIRNNSIFRGREKGKGGRGGGGGGGGGG G...", which represents the protein chain encoded by this ORF. The tool found this ORF in the DNA strand in a positive sense, meaning that the sequence was read normally, from 5' to 3'. This ORF begins at position 18 of the DNA sequence and extends beyond position 1940, covering a large portion of the sequence. The analysis also indicates that the ORF is in frame 3 of reading, meaning that the sequence has been read from the third nucleotide of the chain in the reading frame.

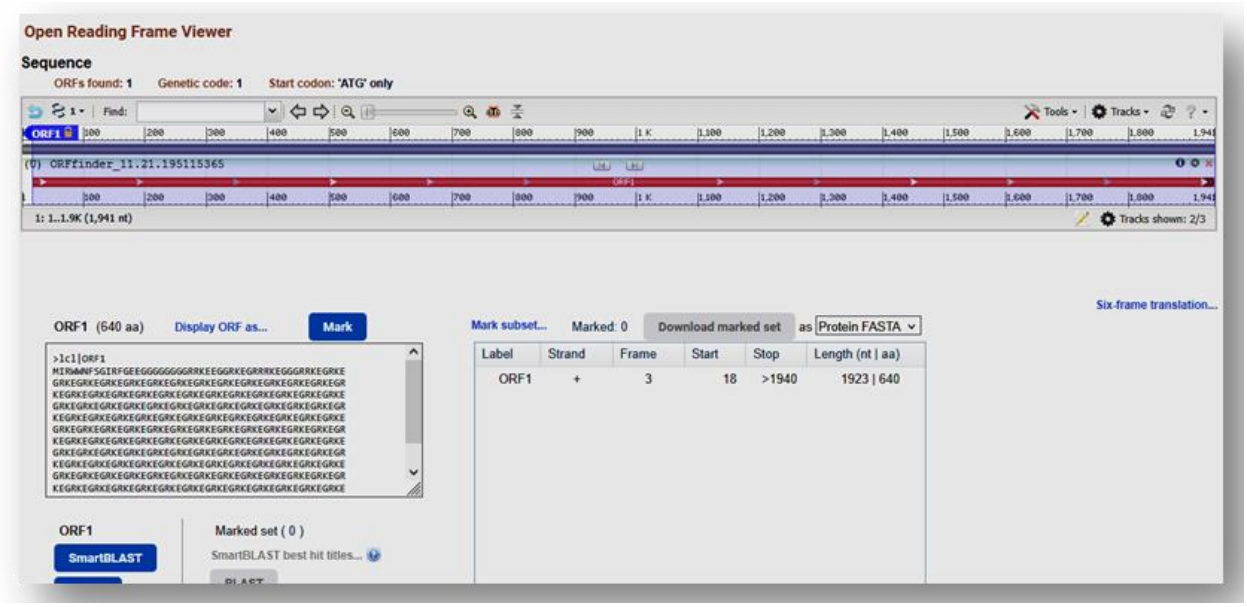


Fig. 6. DNA sequence of *Aspergillus salvadorensis.* Source: NCBI ORF Finder.2025

In general, fungi are eukaryotic organisms belonging to the kingdom *Fungi*, one of the five kingdoms traditionally used for the classification of living beings along with *Animalia*, *Plantae*, *Protista* and *Monera*. Unlike plants, they do not have chlorophyll or photosynthesis, so they depend on the absorption of organic matter from the environment to obtain energy and nutrients, the native species *salvadorensis* depends on the nacascol seed (*Caeselpinia coriaria*). They are distributed in practically all ecosystems on the planet and can manifest themselves both in unicellular forms such as yeasts, and in highly differentiated multicellular structures such as molds and fruiting bodies, with sizes ranging from microscopic organisms to visible macroscopic structures. Its classification is mainly based on morphological and reproductive characteristics, which allows us to distinguish five main groups: *Basidiomycota, Ascomycota, Glomeromycota, Zygomycota and Chytridiomycota*. Basidiomycota are characterized by the formation of basidiocarps, where spores develop on basidia, while numerous Ascomycota are known to produce secondary metabolites, including penicillin (Library, 2025).

A sequence of *A. salvadorensis* is shown in figure (7), presenting a graph of taxonomic distribution of fungi, based on data from genetic sequences. In the graph, the predominant group is the Dikarya (84%), which includes two large divisions of fungi: Ascomycota and Basidiomycota.

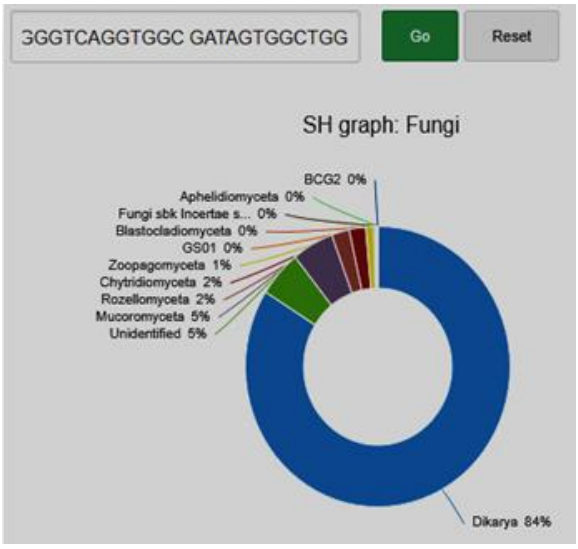


Fig. 7. *Aspergillus salvadorensis* sequence. UNITE. 2025

The synthesis of penicillinase by *Aspergillus salvadorensis* leads to hydrolysis of the β-lactam ring present in antibiotics such as penicillin, which inactivates its antimicrobial activity (Figure 8). The enzymatic degradation of the β-lactam core gives the fungus an intrinsic resistance to these compounds and gives it a competitive advantage in ecological niches where antibiotic β-lactam residues exist. In this context, *A. salvadorensis* can establish and proliferate in microenvironments dominated by susceptible bacteria, displacing or limiting their growth. Although this species is not considered a relevant clinical pathogen, the potential capacity to produce penicillinase represents a biochemical defense mechanism that favors its persistence and adaptation in ecosystems exposed to antibiotic pressure in oxidative stress.

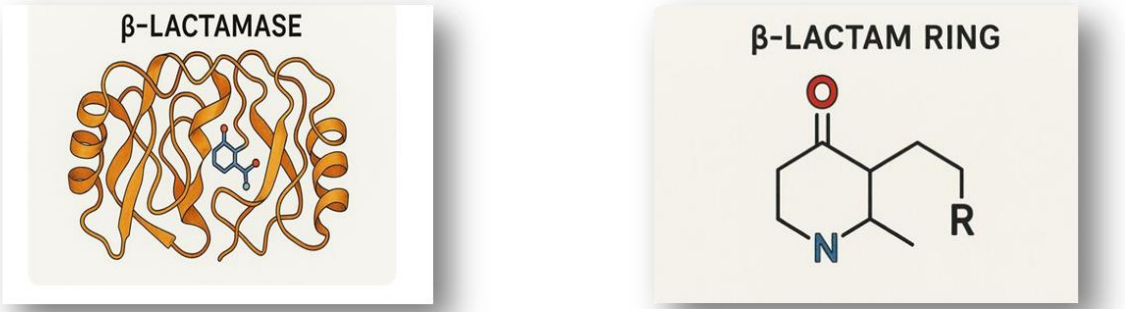


Fig. 8. Image of beta lactamase enzyme and beta-lactam ring.

If *Aspergillus salvadorensis* produces penicillinase in a laboratory environment in small quantities although it was not determined by chemical analysis, this enzyme exerts a direct effect on the microbial dynamics of the culture. Penicillinase released into the extracellular medium breaks the β-lactam ring of penicillin added to the media, thus nullifying their inhibitory capacity on sensitive bacteria. As a result, microorganisms that would normally be stopped by the antibiotic can continue to proliferate due to the inactivation of the compound. This behavior is clearly evidenced in cultures made on Muller Hinton Agar, where the action of penicillinase generates areas of bacterial growth within sectors that, in theory, should show inhibition.

In agar diffusion assays, the presence of *Aspergillus salvadorensis* can form protective zones around its colonies, like a "shield", where sensitive bacteria find an environment with an absence or low amount of penicillin due to the localized action of penicillinase. Similarly, in liquid cultures, enzyme activity can rapidly break down the antibiotic, altering bacterial growth curves and affecting the consistency of microbiological assays.

In summary, the production of penicillinase makes *Aspergillus salvadorensis* an organism capable of interfering with susceptibility tests, selective media, and microbial interaction studies. Their ability to deactivate penicillin significantly modifies the behavior of sensitive bacteria, facilitating their survival, multiplication and spread under conditions that would normally inhibit them non-quantitative qualitative terms.

DISCUSSION

Currently, the study of fungi with medicinal applications is going through a period of extensive growth, driven by the interest in discovering new species with therapeutic potential and in elucidating the mechanisms by which these organisms influence the modulation

of the human immune system. Recent advances in molecular biology, genomics, and biotechnology have allowed the targeted manipulation of various fungal strains, facilitating the optimization of the synthesis and isolation of bioactive metabolites with possible pharmacological use (Frisvad, 2018b).

These species proliferate more frequently in regions with a temperate to cold climate and usually develop in cereals stored under unsuitable humidity and temperature conditions, which favors the synthesis of mycotoxins such as OTA (Bionte, 2025). *A. salvadorensis* grows Latitude: 13.8, Longitude: -88.1, UFI: -1147125, UNI: -1691519, UTM: CA82, JOG: ND16-10 which allows it to adapt to this climate.

Species belonging to the genus *Aspergillus* are distinguished by their remarkable ability to synthesize a wide range of secondary metabolites, including mycotoxins, pigments, alkaloids and other bioactive molecules that contribute to their adaptation, survival and defense against various environmental stressors. The existence of specialized metabolic pathways to produce these compounds reflects the intrinsic potential of the fungus to generate substances with biological relevance. In this sense, recent studies have indicated that a species locally identified in El Salvador, called *Aspergillus salvadorensis*, can produce black pigments of natural origin. At the same time, research focused on fungi with medicinal properties continues to expand, encompassing the identification of new species with potential therapeutic applications, the analysis of their mechanisms of interaction with the human immune system, and the use of advanced molecular biology tools for the genetic optimization of fungal strains aimed at increasing the production of bioactive compounds studies to verify its functionality (Hong, 2023; Vásquez, 2025c).

From a biotechnological perspective, the identification of the *acvA gene* and its associated counterparts with the penicillin biosynthesis pathway opens new opportunities for research in the production of natural antibiotics or semi-synthetic derivatives, as well as for the evolutionary exploration of biosynthetic clusters in species of the genus *Aspergillus*.

The coincidence with UniRef90_A2QZ81 is particularly relevant from a biotechnological perspective, since it is directly associated with the penicillin biosynthesis pathway. This group includes proteins encoded by the *acvA gene*, which is responsible for the production of the enzyme ACV synthetase. The detection of this homology in *Aspergillus salvadorensis* suggests that, like other species of the genus *Aspergillus*, this fungus possesses metabolic pathways specialized in the synthesis of secondary metabolites, including antibiotics, pigments, and other bioactive compounds (Hoffmeister and Keller, 2007).

The production of penicillin in research settings is a carefully regulated biotechnological process that comprises three general phases: fermentation, extraction and purification. This scheme, implemented in stirred flasks or small-scale bioreactors, aims to reproduce in a controlled manner the conditions used in the industrial production of the antibiotic (Bionte, 2025; Roger, 2025).

During the fermentation stage, the main purpose is to promote the formation of penicillin, a secondary metabolite produced by certain fungi. For this purpose, strains of *Aspergillus nidulans* or, more frequently, *Penicillium chrysogenum*, historically recognized as the fundamental microorganism in obtaining this antibiotic, are usually used (Brakhage,1998). The fungus develops in a nutritious liquid medium that incorporates appropriate sources of carbon – such as lactose or combinations of this with other sugars and complex sources of nitrogen derived from plant materials or microbial extracts, the nacascol seed precisely contains these nutrients, which makes it exclusive as a source of carbon because the seed has carbohydrates, proteins as a source of hydrogen for energy and growth, lipids as energy (Brakhage, 1998; Schüffler, 2018; Berdy, 2005).

When a particular type of penicillin, such as penicillin G, is required, a compound that acts as a precursor of the side chain is added to the culture; in this case, phenylacetic acid directs the metabolic pathway towards the generation of the corresponding antibiotic (Demain and Elander, 1999; De Sales, 2025).

CONCLUSION

The *acvA* gene in the DNA chain of *Aspergillus salvadorensis* was reported by MACROGEN INC SOUTH KOREA, so it is concluded that it is probably a precursor of the synthesis of Penicillin in rapid qualitative tests and is present, as recorded in the files of the microbiology department of the Faculty of Medicine. In genomic analysis, the identification of homologous sequences to the UniRef90_A2QZ81 cluster supports the presence of the *acvA* gene (also known as *pcbAB*), responsible for encoding the *ACV* synthetase enzyme. This gene is a fundamental element of the biosynthetic penicillin cluster, therefore if it is identified in the genus Aspergillus of other species, it is indicative to perform biochemical tests to verify quantification. Tukey's test was applied to compare the means of the two treatments studied. The difference observed between the means was 0.006. It is concluded that there is no significant difference between treatments. This result is consistent with the ANOVA, which also showed a non-significant difference between the groups in *Staphylococcus aureus* and *Pseudomona sp*.

CONFLICT OF INTEREST

The author declares no conflicts of interest.

REFERENCES

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for evaluating antimicrobial activity in vitro: A review. J. Pharm. Anal., 6(2): 71–79.

Berdy, J., 2005. Bioactive microbial metabolites. J. Antibiot., 58(1): 1-26.

Bionte. 2025. Hongos y micotoxinas: *Penicillium* spp. Online.

Brakhage, A.A., 1998. Molecular regulation of β-lactam biosynthesis in filamentous fungi. Microbiol. Mol. Biol. Rev., 62(3): 547–585.

De Sales, T., 2025. Fungi. En: *Microbiology*.

Demain, A.L., Adrio, J.L., 2008. Contributions of microorganisms to industrial biology. Mol. Biotechnol., 38(1): 41-55.

Demain, A.L., Elander, R.P., 1999. The β-lactam antibiotics: Past, present, and future. Antonie Van Leeuwenhoek. 75(1–2): 5–19.

Elander, R.P., 2003. Industrial production of β-lactam antibiotics. Appl. Microbiol. Biotechnol., 61(5): 385–392.

Falk, L.A., 1999. The history of penicillin. JAMA., 281(20): 2049–2051. doi:10.1001/jama.281.20.2049

Fleming, A., 1929. On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of *B. influenzae*. Br. J. Exp. Pathol., 10: 226–236.

Frisvad, J.C., Houbraken, J., Samson, R.A., 2018a. Taxonomy, chemodiversity, and chemoconsistency of *Aspergillus*, *Penicillium*, and *Talaromyces*. Front. Microbiol., 9: 1286. doi:10.3389/fmicb.2018.01286

Frisvad, J.C., Larsen, T.O., Thrane, U., Meijer, M., Houbraken, J., 2018b. Fungal secondary metabolite profiling for drug discovery. Nat. Prod. Rep., 35(5): 474–490. doi:10.1039/C8NP00002J

Hoffmeister, D., Keller, N.P., 2007. Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat. Product Rep., 24(2): 393-416.

Hong, K.H., Cho, H., Shin, E.T., Lee, Y.H., Frisvad, J.C., 2023. Secondary metabolite pathways in *Aspergillus* sect. Nigri. Nat.

Prod. Rep., 40(2):259–287. doi:10.1039/d1np00074h

Houbraken, J., Samson, R.A., Yilmaz, N., 2016. Taxonomy of Aspergillus, Penicillium and Talaromyces and its significance for biotechnology. 1-15. Caister Academic Press, Wymondham: UK.

Iqbal, M.N., Iqbal, I., Yunus, F.N., Muhammad, A., Shahzad, M.I., 2021. A Review of Mycotoxins Produced by Fruit Spoilage Fungi. PSM Biol. Res., 6(2): 46-49.

Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery. Nat. Rev. Microbiol., 17(3): 167–180.

Library. 2025. Taxonomía e identificación de hongos del género *Penicillium*. Online.

Ligon, B.L., 2004. Penicillin: its discovery and early development. InSeminars in pediatric infectious diseases. Jan 1 (Vol. 15, No. 1, pp. 52-57). WB Saunders.

Liras, P., Martín, J.F., 2006. Gene clusters for beta-lactam antibiotics and control of their expression: why have clusters evolved, and from where did they originate?. Int. Microbiol., 9(1): 9.

Lysakova, V., Krasnopolskaya, L., Yarina, M., Ziangirova, M., 2024. Antibacterial and antifungal activity of metabolites from basidiomycetes: A review. Antibiotics., 13(11): 1026.

MacCabe, A.P., Riach, M.B., Unkles, S.E., Kinghorn, J.R., 1990. The Aspergillus nidulans npeA locus consists of three contiguous genes required for penicillin biosynthesis. The EMBO J., 9(1): 279.

Macrogen. Genomic sequencing report: *Aspergillus salvadorensis*. Korea; 2025.

Martín, J.F., 2000. Molecular control of expression of penicillin biosynthesis genes in fungi: regulatory proteins interact with a bidirectional promoter region. J. Bacteriol., 182(9): 2355-62.

Moore, D., Ahmadjian, V., 2025. Fungus: Definition & facts. Encyclopaedia Britannica.

NCBI, 2025. National Center for Biotechnology Information. BioProject PRJNA1306032, PRJNA1303219.

NTP, 2021. National Toxicology Program. 15th Report on Carcinogens: Ochratoxin A. doi:10.22427/NTP-OTHER-1003

Perrone, G., Gallo, A., 2016. Aspergillus species and their associated mycotoxins. Mycotoxigenic fungi: Methods and protocols. 7: 33-49.

Rogers, K., Britannica Editors., 2025. *Penicillium chrysogenum*. Encyclopaedia Britannica.

Samson, R.A., Visagie, C.M., Houbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H., Perrone, G., Seifert, K.A., Susca, A., Tanney, J.B., Varga, J., Kocsubé, S., Szigeti, G., Yaguchi, T., Frisvad, J.C., 2014. Phylogeny, identification and nomenclature of the genus Aspergillus. Stud. Mycol., 78: 141-73. doi: 10.1016/j.simyco.2014.07.004.

Schüffler, A., 2018. Secondary metabolites of basidiomycetes. InPhysiology and Genetics: selected Basic and Applied aspects (pp. 231-275). Cham: Springer International Publishing.

Vásquez, A., 2025a. Prevalence of Secondary Metabolites Target Carcinogenic Clusters in the Circular DNA Sequence of *Aspergillus salvadorensis* to Aflatoxins. PSM Microbiol., 10(1): 177-96.

Vásquez, A., 2025b. *Aspergillus salvadorensis* sp. nov. Research Gate. https://www.researchgate.net/publication/397990403_Aspergillus_salvadorensis_sp

_nov/ repositorio UES https://repositorio.ues.edu.sv/server/api/core/bitstreams/f5632779-b8c4-4172-be02-557a9294a15d/content

Vásquez, A., 2025c. Characterization of *Aspergillus salvadorensis* Isolated from *Caeselpinia coriaria* Seed, El Salvador. IKR J. Agric. Biosci., 1(4): 189-205.

Waktola, D., 2024. Antibacterial metabolites from fungi. Afr. J. Microbiol. Res., 18(4): 155–165.

Watson, J.D., et al. 2013. Molecular Biology of the Gene. 7th ed. Pearson.

Zhou, L.W., May, T.W., 2023. Fungal taxonomy: current status and research agendas for the interdisciplinary and globalisation era. Mycol., 14(1): 52-9.