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Research Article

Phenotypic and Genotypic Characterization of *Aspergillus uessalvadorensis* in an Organic Strain Discovered at the University of El Salvador 2006 - 2024

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Abstract

Morphological characterization and molecular DNA techniques allowed the identification of the *Aspergillus* sample sent to MACROGEN SOUTH KOREA. *Objective*: To perform phenotypic and genotypic characterization of the genus Aspergillus by the Next-Generation Sequencing method (NGS). *Methodology*: The type of study is exploratory and experimental. It was carried out in two phases: first in the collection of seeds of *Caesalpinia coriaria* and second an initial macroscopic and microscopic characterization of the isolation of *Aspergillus was carried out* in 2006, a notarial act was carried out in 2007, studies of simple microscopy and scanning electron microscopy and PCR of the fungus found in Mexico 2008 and published in the journal La Universidad in 2008, then the extraction of gDNA, qPCR, cDNA was performed in 2024 at MACROGEN INC. by Metagenome Shotgun Sequencing Reports. *Results*: The gDNA genome was extracted obtaining a maximum concentration of 12.297 ng/ul, volume 30 ul, total amount 0.369 and DIN 6.4 maximum level 8732 sample intensity for 15000 bp, the quantum qDNA was obtained at 624 bp at a concentration of 103.24 nM and 41.87 ng/ul and cDNA. From the gDNA extraction of the TapeStation gDNA Screen, a maximum concentration of 12.297 ng/ul, volume 30 ul, total quantity 0.369 and DIN 6.4 maximum level 8732 sample intensity for 15000 bp in quality control was obtained. qPCR 624 bp were obtained at a concentration of 103.24 nM and 41.87 ng/ul with the TruSeq Nano DNA library (350_META). cDNA library 33 library kits were used. Total, of bases obtained were 11,705,895,990 bp, total, of readings were 77,522,490. GC nucleotide content % 49.7 and AT % 50.30, GC base content was 49.7% and AT was 50.3, Metric base content Q 20: 95.1 and Q 30: 88.3, Q30 cycle data quality high value. FASTQ and FASTA formats were used for encoding and full base-pair sequencing. Raw data Value. 38,761.245 N, adapter quality and trimming. (Quality and adapter trim.) was 32,535,420, Elimination of contaminants was 30,961,740. From the Krona taxonomy, *Aspergillus* was reported. Heatmaps specie report varieties. *Conclusions*: *Aspergillus* sp was found by the NGS sequencing method *with a variety of species*, in previous studies in 2006 it was named *the species uessalvadorensis*.

Keywords

Aspergillus sp, Nacascol, Uessalvadorensis

1. Introduction

The initial study of the *Aspergillus* fungus was first described in 1729 by the Italian biologist Antonio Micheli in his work Nova Plantarum who was born in Florence, Italy, in 1697 [15]. Today, it has generated a lot of interest not only

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because of its implications on health but also in the food, chemical and biological industries. General research began on a natural plant well known in our environment at the national level known as the Nacascol seed, widely used in pottery and leather tanning in the northern part of the country. This seed has an inert particularity that requires the presence of a fungus of the genus *Aspergillus sp* and the fungus devours the seed as a food substrate by piercing it until it is pulverized, so that it can be dyed together with the properties of the clay dyes the vessels black-brown and tanning of the skin of the cattle, The tannins when fermented can extract gallic acid and glucose. In addition, the vessels give the special characteristic of black clay, which gives a colonial and beautiful appearance to the vessels. When looking at the pots, the brown tone is from the tannin of the seed and the black comes from the mushroom plus iron contained in the clay gives it the intense black tone. In general, species of the genus *Aspergillus* are found as saprophytes deposited in organic material such as plants, seeds or soil. Their temperature is adaptive, from a minimum of 5 ℃ to 60 ℃ in other species, although rare they withstand temperatures of more than 100 to 300 ℃. *Aspergillus* is currently an amorphous genus, comprising between 260 and 837 species [20, 23]. Such is the diversity of species that with the use of modern technologies such as PCR. Sequencing has made it possible to facilitate its study in some cases, as long as an adequate sequential database is available and the primers according to species are available to mix the base pairs. The pigmentation of fungi is due to the synthesis of various types of pigments and chromophore molecules such as carotenoids, melanins, flavins, phenazines, quinones among others. [22-33]. Melanin pigments have a primary role in protecting fungal spores against temperature increases, radiation, and desiccation. [38] Melanins are present in most fungi, being found in almost all pathogenic fungi. [53] The natural habitat of *Aspergillus* species is hay, seeds and compost. Justification. A complete study was carried out on the extraction and identification of DNA from the genus *Aspergillus* to determine what type of species circulate in our country. The research began in 2006 with phenotyping studies in the laboratories of the Microbiology department of the Faculty of Medicine of the University of El Salvador, then in 2008 in the scanning electron microscopy laboratories at CENSALUD and then in Mexico preliminary molecular PCR results, then in 2024 in the laboratories of MACROGEN INC in South Korea the complete study of extraction, quantification and sequencing of g,q,c,ds,seqDNA in its molecular analysis of the genus *Aspergillus* to determine the type of native species circulating in the northern part of our country. Objective: To perform phenotypic and genotypic characterization by the Next-Generation Sequencing method of the genus Aspergillus. Hypothesis: H1. Phenotypic and genotypic characterization of DNA helps identify species of microorganisms in the environment. H0 Phenotypic and genotypic characterization of DNA does not help identify species of microorganisms in the environment.

2. Material and Methods

The type of study is exploratory with a significance level of 95% and an estimation error of 0.05%. From the fungus *Aspergillus.* 1. Characterization. Its study was developed in two phases: I: Phenotypic Characterization. Five batches of *Caeselpinia coriaria* seeds were collected from the northern zone, purification and selection of the seeds according to their appearance and color. The inoculums were then made in plates and test tubes in Sabouraud culture medium. Growth was observed at 48 hours in the Microbiology laboratory at 40x and 100x under a simple light microscope over weeks with a comparison sheet. It was taken to the laboratory for scanning microscopy observation of *Aspergillus* by taking electron microphotographs at CENSALUD. In Mexico, a preliminary PCR was carried out. Phenotypic characterization was completed. II: Genotypic Characterization. Three samples, one pure strain in glass tube and two more samples, one sample was DNA and the other simple, were sent to South Korea in the laboratories of MACROGEN INC for the extraction, analysis and identification of g,q,c,ds,seqDNA. Preparation of the spore suspension and microculture. A spore suspension was prepared from culture where the conidia or spores were identified where they were extracted by simple method under the microscope and reseeded, which was used to perform the inoculum in the Sabouraud culture medium in tube and plate, the fungus was sown in a 250 ml Erlenmeyer flask, to which 50 ml of the medium was previously added, incubated for 48 hrs at 28 ℃. Subsequently, 100 ml of distilled water at room temperature was added and carefully stirred for 5 min with the help of a magnetic stirrer. It was also passed into the tube in Saboraud and stirred for homogenization to ensure that the spores are well suspended. If the spores tend to settle, it may be helpful to use a mixer or shake manually. Spore counting was performed with a Neubauer chamber for adequate concentration required. [10]. Microculture: It was carried out at CENSALUD and consisted of preparing a sterile wet chamber: a petri dish, at the bottom of which sterile water is placed and a V-shaped glass rod, on which the slide is placed, and on top of it the block of the Saboraud culture medium. The sample is inoculated on the block in four quadrants, by means of the L-shaped platinum loop on the block of the medium already inoculated, a coverslip is placed and incubated for 7 days at 25 ℃. Once the incubation period is over, the coverslip is removed and placed on a sheet with one or two drops of Lactophenol Blue and the characteristic structures of the isolated species are observed under a microscope with a 100x objective. [12] 2. Sampling and collection area. The fungus was isolated from nacascol seeds from the northern zone. The brown-black seeds were identified, collected and stored in airtight bags for subsequent analysis at room temperature. It does not require special measures for transport and conservation. 3. Morphological characterization of fungal samples. The morphological characterization was carried out in cultures in Saboraud and incubated at 37 ℃ for

72 hours in aerobic tubes and then at room temperature x 7 days. With simple microscopic observation on cotton blue lactophenol at 10 x, 40 x and 100x, and then sealed with a coverslip. The visualization of the structures was carried out under an optical microscope. Taxonomic affiliation was used to determine the online database. 4. Sample isolation and purification. Seed cleaning was not used in the plant material. It was scraped and the material was removed and deposited in ASD (Sabouraud dextrose agar) culture media for isolation and differentiation. The culture plates and tubes were incubated at temperature for one week. This process was repeated until pure isolates were obtained. Previously isolated samples were inoculated in 150 mL of PW liquid culture medium (peptone, water, and nutrient broth) in dish and tubes and incubated at 37 ℃ in 48 hours and at room temperature between 20 and 25 ℃. The biomass was dried at 42 ℃ for two days and left at room temperature at 25 ℃ for 3 weeks until further analysis. 5. DNA method. In general, methods for DNA sequencing were used. The Sanger sequencing method was used by the enzyme chain termination method and automatic sequencing. Sequencing is carried out in three steps: 1. Perform the synthesis of new DNA fragments. 2. Separate the fragments by electrophoresis and finally 3. Identify nucleotides to determine the sequence. In terms of microbiology, molecular study can be performed by sequencing small fragments of deoxyribonucleic acid (DNA) that have been previously amplified, or by sequencing all previously fragmented DNA randomly. Sequencing through *Illumina* is basically characterized by the execution of the following processes: a. The amplification of DNA fragments for the generation of clusters (colonies of the same fragment) is performed by the PCR method bridge. b. Base detection in sequencing is done through fluorescent markers. Therefore, the Illumina platform allows DNA fragment sequencing at both ends. The fragment is sequenced amplified by both ends rather than a single end. Including your gDNA. qDNA, dsDNA, cDNA and seqDNA and then analyze them in computer packages. [35] 6. gDNA extraction. gDNA genes are currently one of the most studied forms in the fungal genome, mainly for the detection and identification of species through molecular biology. In the preparation of the above sample and its extraction method has as its main objective that it must release the intracellular DNA, breaking the wall of the fungal cell or mycelium, the cell membrane and the nuclear membrane by heat at temperature controlled by the thermal cycler or another way. In addition, it must concentrate the target molecules of DNA that may be present in small quantities and must be purified by removing traces of contaminants, proteins, foreign RNA remains, without degrading the nucleic acid. For the rupture of fungal cells it can be performed by different methods: such as chemical, enzymatic or mechanical [39], which are the most common, but it will depend on the laboratory in its extraction method. [35] 7. qPCR technique. Called quantitative DNA measured by fluorometry that captures nucleotides. After having met the requirements for gDNA extraction in quality control, the qDNA was fragmented and quantified by degrading it. The DNA quality control method in MACROGEN were: 1. Quantity of DNA: Performed by the QuantiFluor® dsDNA System method using the Victor Nivo multimode microplate reader. Macrogen quantifies the starting genomic material using fluorescence-based quantification. using specific double-stranded DNA dyes, this method quantifies dsDNA more accurately than the UV spectrometer, even in the presence of common contaminants. The species of the genus Aspergillus spp in their genomic evolution have been variable in their size, in which they have an average range of genome size of 27 to 39 Mb or more and an average number of genes between 9000 to 14000 genes. The one with the largest size is A. sojae 39.5 and the one with the highest number of genes is *A.niger* con 14,165. [5-8] 8. Quality per cycle. The term Quality by Cycle refers to the evaluation of the quality of sequencing readings based on each sequencing cycle. The sequencing process is carried out in cycles. During each cycle, a base is added to the DNA fragment and the embedded base is detected. It is sequenced base by base in a series of cycles. In each cycle, a base (A, T, C, or G) is incorporated into the DNA strand, and a fluorophore or optical signal detects the added base. This process is repeated multiple times, and in each cycle it is detected and recorded on a basis. [35]

3. Results and Analysis

3.1. Phenotypic Characterization of Aspergillus from El Salvador

From the tree called NACASCOL whose scientific name is *Caeselpinia coriaria*, of the family *Caesalpnieaceae*, of the genus *Caesalpinia*, a leguminous plant with a stem from 3 to 11 meters high, with leaves in pairs pinnae 5 to 10 cm long, each with more than 10 leaflets 4 to 8 mm long and 2 mm wide, The seeds are approximately 3 to 4 cm long, apex rounded, brown seeds with a black appearance, from which grows a fungus whose resemblance is to a possible *Aspergillus sp*. The *Caeselpinia coriaria* tree contains tannins, triterpenes, glycosides, and flavonoids. The fungus reproduces exclusively in the seeds of the Nacascol tree, from which it is prepared for the extraction and preparation of the dye and then cultivated in vitro for the production of the dye by Salvadoran artisans. [51]. The fungus found in the seeds is described as follows: Sterygmas. The head of the conidia is black, smooth conidiophore 1 to 4 μm long with moderate conidia or internal spores of 1 to 3 microns, brown to black. It is a hyaline, saprophytic filamentous fungus, belonging to the phylum Ascomycota. Macroscopic characteristics: When cultured in special Saboraud medium they produce a black coloration with pigment production in the tube in the posterior region in the tubes within 7 days. In plate and tube Saboraud agar culture: the colony its mycelium is white with a columnar appearance and then turns black. The texture of the colognes,

they look like cotton or velvet, there are no sclerotes. The reverse of the colony is black or pigmented black depending on the oxidizing agent, low mycelium height, appearance of the colony is dusty black. It is a filamentous fungus. Microscopic characteristics: The conidiophore of *Aspergillus* has three distinct parts: a terminal globose vesicle with a uniseriate fialid without metal, a tubular stipe, and the foot cell that joins the conidiophore with the mycelium. Conidiophores are smooth-walled and rough and are grouped together to form a compact, hyaline or pigmented mass and are 3 to 5 µm long and 15 to 20 µm in diameter. Hundreds of spores or conidia are observed inside. The spore is spherical and irregular, aseptate, ameroporous, colored and dark of the columnar feospore type. The vesicle is globose with 52-66 µm in diameter with a stipe length of 100-300 µm, a stipe width of 2-2 µm, irregular ornamentation, and produces phylalides around it. The phylalides are monoseriate, the primary branches are 30 µm long, short and 8 microns long, from which the conidia sprout, which are globose and rough 4 to 5 µm in diameter, brown brown or brown to black. The diameter of the colony is 9-10 µm, the conidia are columnar and have a length of 3-5 µm and a width of 1.96-2 µm. Smooth conidia heads with a round and irregular wall, arranged in a column; smooth, pronounced, thin-walled stipes, brown to black; no columella is observed; there are abundant conidia that detach from the head; It has a row of phylalides. The sporangium is a simple globose peridial structure, the sterygma is black. Mature brown conidia are spherical with abundant spiny-like projections throughout the periphery; and the central ones are few, forming spicules in number greater than ten. The conidia have a spherical appearance in the form of a mallet with spicules, from the ends emerge long filaments arranged in the form of linear chains that come out of the protuberances. The entire structure forms a solid shell. Immature asexual spores are spherical, thin-walled, colorless, large in size, which then fill in to form internal brown masses. The conidiophore is long, smooth, uniseriate, with abundant spores. In general, the spore is elongated and irregular, aseptate, ameroporous, colored, and dark of the type feospora, large, with a diameter of 1 to 3 µm; Hyphae are not septate with continuous mycelium. The head is not radiated, it is columnar, it is uniserial, from which the conidia are detached, it grows at room temperature of 37 ℃. Immature asexual conidia/spores are irregular with a thin, colorless wall of large size, which then fill in forming brown inner masses, the appearance of the colony is dusty black. The mycelium is short or flattened and thin and black as it ages. This species of *Aspergillus* found, it is suggested that given its morphological characterization it is another found in El Salvador deduced by the international taxonomic classification. When comparing both the *niger* and the discovered species, the vesicle of the *niger species* is irradiated with round conidia and the other discovered is shaped like an elongated mallet without irradiation. At 100x it can be observed that the conidia of the *niger* are round with thick walls and a smooth center biseriate, with metula and radiated [46]. The one of the new species has spicules coming out of the armor. Both conidia can be observed, in which it is highlighted that the conidia of the new species are not stained with cotton blue lactophenol and those of *niger* are [51]. *Aspergillus* has asexual reproduction because it has conidia formation, at first the mycelium is white and then after weeks at room temperature it changes to black, with progressive increase in brown sporulation. These grow at the ends of the phylalids. Due to the presence of pigments, it looks black in color. The fungus of the genus *Aspergillus spp* has the particularity of being mycotoxin producers. Mycotoxins are secondary metabolites produced and secreted by the fungus during the degradation process of organic matter, as a defense mechanism against other microorganisms. [14-43]. The hyphae continue their way in a codinidophore that at its terminal end ends in a globose conidial head from which the phyllids emerge and the spores or conidia are detached to return to their cycle. Each hyphae, at its end, forms globose conidial heads that can produce more than 500,000 conidia. [31]. These conidia at 100x have a rough appearance like pectorals, thick wall with brown spicules projecting outward, filled and whole that are not colored with cotton blue lactophenol. From the base of the hyphae, each conidia is pushed into the conidiophore to the end until it reaches the vesicle where it accumulates, exerting a mechanical effect of pressure outwards from where it emerges from the phylids to the outside, this is constant over and over again. Hyphae are filamentous cylindrical structures that form the body of multicellular fungi. They are made up of a row of elongated cells wrapped by a chitinous cell wall. The cells that make it up may or may not be separated from each other by a transverse cell wall called septa. These conidia are considered infectious and constitute the starting point for the development of the mycelium of the fungus. Uniseriate hyphae are usually transparent where the spores are visible. It is a fungus that produces mycotoxins that are considered as secondary metabolites produced and secreted by the fungus during the process of degradation of organic matter, as a defense mechanism against other microorganisms that produce aflatoxins B1 and M1 that are carcinogenic, others also associate it with mycotoxins or ochratoxins that are secondary metabolites of the strains of *Aspergillus and Penicillium.* [9-11, 34-40] mainly *Aspergillus flavus* with aflatoxins B1, B2, G1 and G2 and *Asperillus parasiticus* both related to Pulmonary Aspergilloma or Lung Abscess or also various cancers of the liver, kidney, pancreas, colorectal. [9-50]. The fungus under study produces mycotoxins and bioactive peptides related to the lung diseases Aspergillosis. The growth in the tube with Saboroud after weeks becomes flat velvety black. In a petri dish, when the colony is old, the conidia chains detach from the head, resembling a sharp fall, exposing only the gallbladder surrounded by an almost black halo. When viewed under a 40 x microscope, more than 20-30 abundant brown conidia are observed per field that are not stained with cotton blue lactophenol. As it has more than 1 to 2 months on Agar saboraud,

it dries out with the medium and the entire colony shrinks, so the fungus does not sporulate but releases the spore. But conidia are viable, so if a pure crop is carried out in a new reseeding, the fungus grows again in optimal conditions. In the petri dish, growth is similar except that at first the mycelium is white and then turns black. No sclerotes, which is characteristic of the *Niger*, are observed, but in our case it does not occur. Over time, more than a month, it turns velvety black. Figure 1 shows the phenotypic characterization described above.

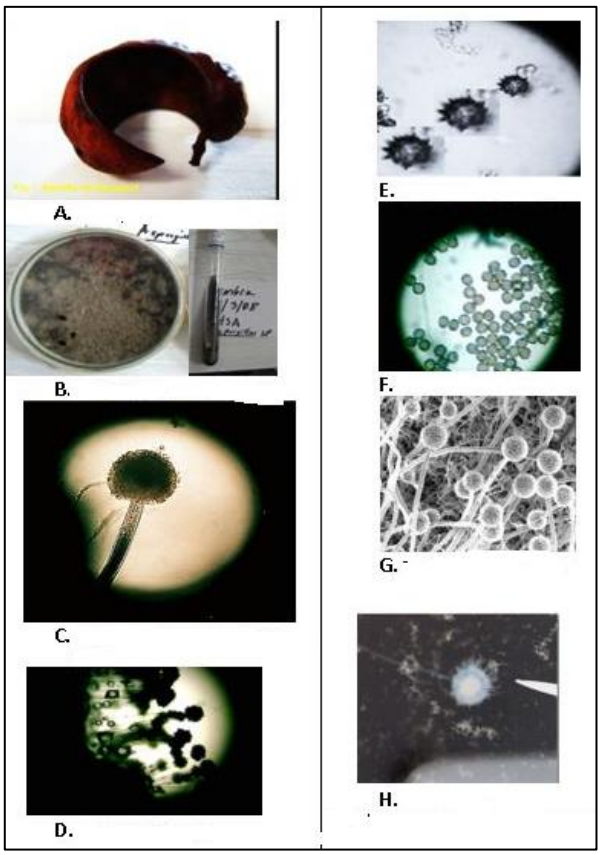

Figure 1. *Aspergillus uessalvadorensis. Phenotypic characterization. A.seem Nacascol, B. culture and tube Saboraud, C. Aspergillus conidial heads 100x, D. Microculture conidial head 100x, E. Conidia 100x, expanded, F. Conidia 100x, G y H. conidiophorus and vesicles conidial heads.*

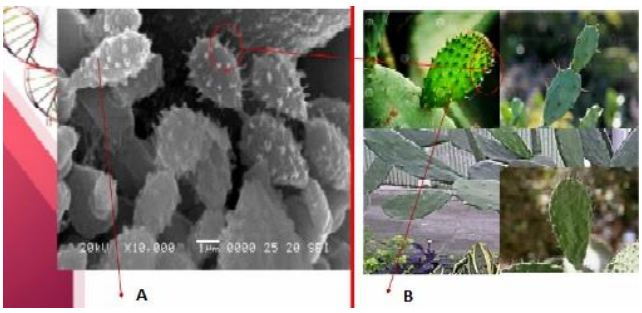

Figure 2. *Comparison of species with natural cactus plant. A. Aspergillus uessalvadorensis. B. Cactus plant.*

In Figure 2, Comparison of the species with spicules conidia with the natural cactus plant that also observed spicules. These give it heat resistance and provide moisture. They can survive temperatures above 60-100 ℃ or more. The spines or spicules protect it from heat by providing moisture. In Figure 3, By the laboratory scanning electron microscopy method, it was found that two weeks after culture, conidia emerge from the uniseriate phylalides in the vesicle column. There was a rapid growth of sporulation within five minutes, within an hour there were millions of spores in 10 fields. The rough hyaline conidiophore with an ogl head and hyphae is observed, in Figure 1 (C) elongation of the hyphae accumulated with filament and branching is observed. There are racket-shaped conidia Figure 1 (G). This *Aspergillus* is in a position to favor high temperatures since relative humidity stops the growth of fungi that produce these toxins temperature as aflatoxins, when exposed to heat it grows and is heat-resistant. Fungi are formed by a wall composed of 80% carbohydrates that constitutes 30% of the dry weight, this wall is rigid which allows it to be protected. Its chemical composition is based on proteins and glycoproteins in 10%, lipids in 8%, minerals such as calcium, magnesium and phosphorus. They have components of chitin, chitosans, peptidoglycans, and mannans. In the laboratory, chitin is softened with 40% potassium hydroxide for morphological study. [18]. Mushrooms do not have cellulose in most of them, unlike vegetables. *Aspergillus* has a very simple biological cycle, in which spores are formed for reproduction and after germination hyphae are formed, which will be the invasive forms of the fungus. The spores are found in the angiospore, so they have a high rate of sporulation and therefore their concentration in the environment is high. The size of the spores or conidia is 0.2 to 3.5 microns. [1-4]. The pulmonary alveolus is a sac that measures approximately 0.2 to 0.5 mm in diameter, it is smaller than the alveoli so they can be easily inhaled. They resist high temperatures due to their hard shell, some contain melanin that protects them from the heat and can travel miles away to other areas and colonize. They grow quickly in less than 5 days and have already matured. Figure 4 shows the heads of the A.niger and A.uessalvadorensis *species*, in which the differences of *A.niger are observed*, their head is round, radiated and biserialized with metula, while *A.uessalvadorensis* they have the shape of an elongated columnar mallet at their end uniserial, without cetula. Multiple species and varieties of the genus *Aspergillus* circulate around the world, Rapper and Fennell qualify around 900 species of *Aspergillus are known*, which in 18 groups, of which only 12 are related to human diseases: *Aspergillus fumigatus (85%), A. flavus (5-10%), A. niger (2-3%), A. terreus (2-3%), A. versicolor, A. nidulans, A. glaucus, A. clavatus, A. cervinus, A. candidus, A. flavipes, and A. ustus* et al. [2-42]. In general, the genus *Aspergillus* has between 30-40 megabases (Mb), which is equivalent to about 30-40 million base pairs (bp) to be considered in a sequencing. [2-12, 49].

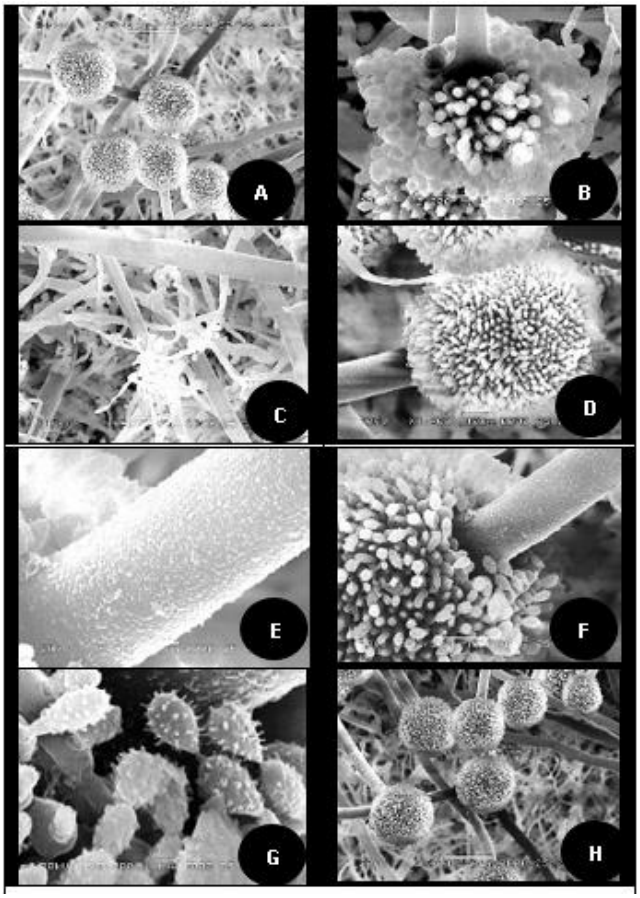

Figure 3. *Aspergillus uessalvadorensis Scanning electron microphotography. Courtesy of CENSALUD by Dr. Vianney de Abrego. A.Morphology Aspergillus, B. vesicle, C: Zapata, elongation of hyphae with filament and branching D. Head of conidia, E. rough codiophore, F. globose and fialide vesicle, G. conidia, H. head of columnar conidials and conidophore.*

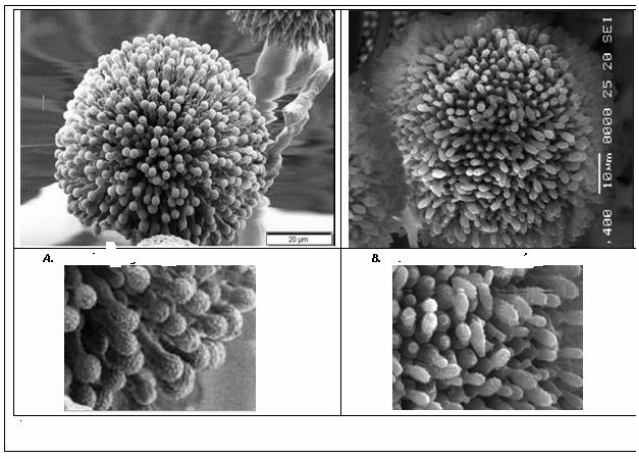

Figure 4. *Heads: radial globose vesicle of the species A. niger and B columnar globose vesicle of A. uessalvadorensis.*

3.2. Genotypic Characterization

1) PCR preliminar

In Figure 5. The PCR of the sample of the species is observed, in which it stands out in the first place that it is of the genus *Aspergillus spp*. In agarose gel, PCR products or amplicons are represented by bands of a specific size and compared to a known molecular weight marker to determine the specificity of the reaction. PB = number of base pairs. Several samples are observed and several species comparable to the study sample are highlighted.

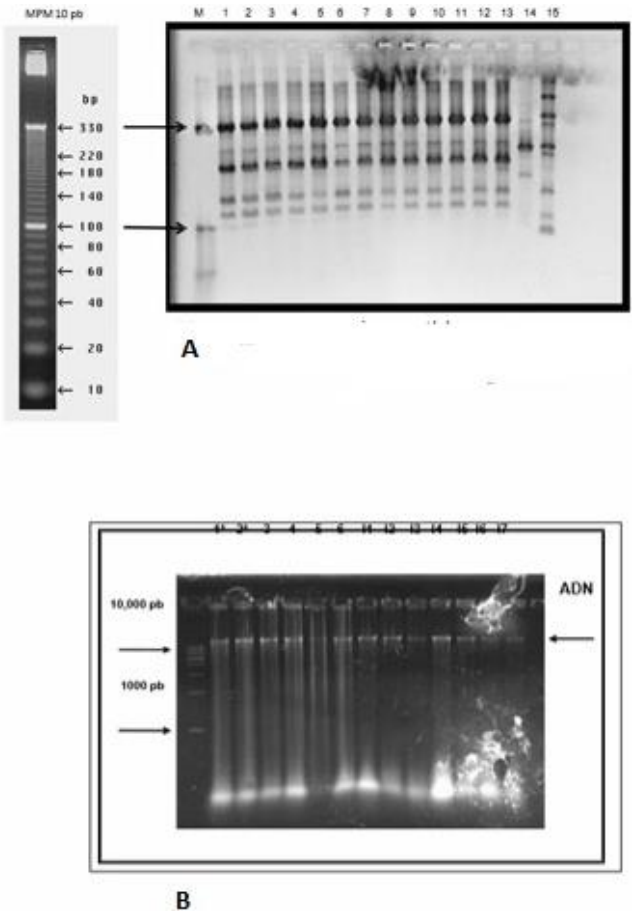

Figure 5. *PCR sample of different species. El Salvador. A. Aspergillus uessalvadorensis. B. Others species. Source: Institute of Molecular Biology. México.*

2) PCR

3) gDNA extraction.

4) gDNA extraction Aspergillus

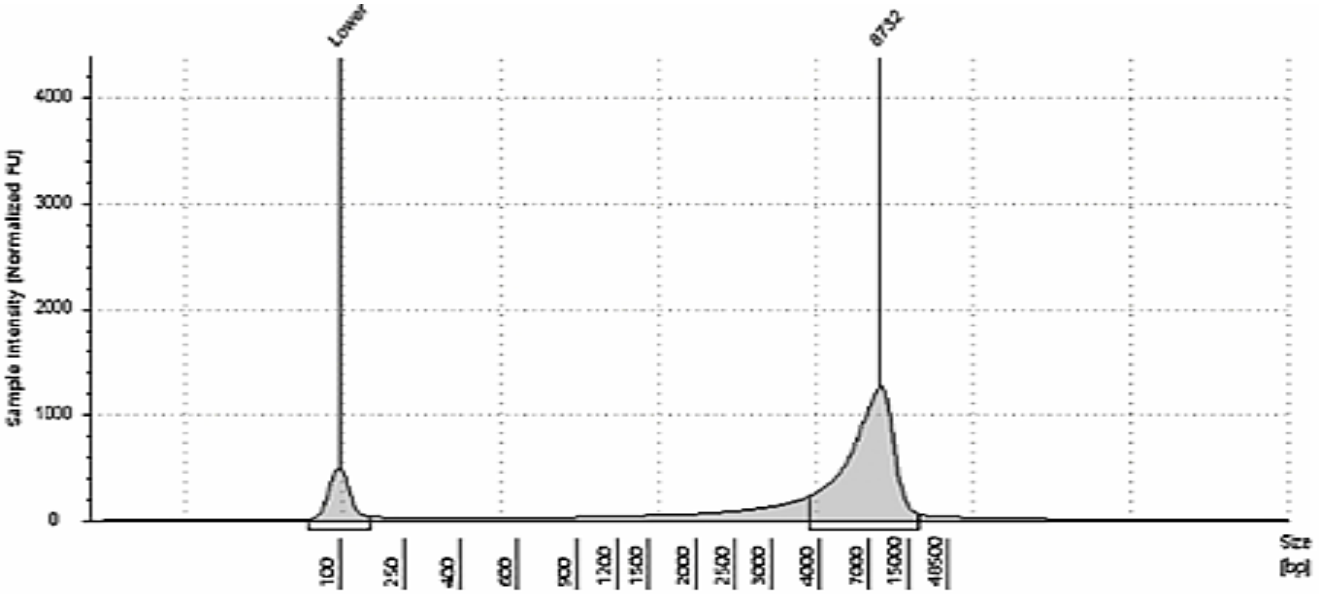

Figure 6. *Aspergillus sp gDNA extraction MACROGEN INC. 2024.*

Analysis. In Figure 6. The extraction of gDNA from the TapeStation gDNA Screen Tape was observed, obtaining a maximum concentration of 12.297 ng/ul, volume 30 ul, total quantity 0.369 and DIN 6.4 maximum level 8732 sample intensity for 15000 bp in quality control. This indicates that the sequencing and identification of the fungus may continue. In the first corner, the sample of its load was insufficient. It refers to DNA extracted from cells or mycelium, which contains the complete genetic information of an organism based on the quantity, quality, or characteristics of the total DNA extracted from a sample before sequencing. Primers should be specially designed to ensure high specificity and generate amplicons ranging in size from 100 to 150 bp. A high concentration: Indicates a good amount of extracted DNA, which is essential for sequencing. If there is not enough DNA, the quality of the sequencing can be compromised. Low concentration: It may indicate problems with extraction or that the sample has a low amount of DNA. Genomic gDNA is the DNA that contains the complete genetic information of an organism, including all the genes and regulatory regions present in its genome.

5) qPCR technique.

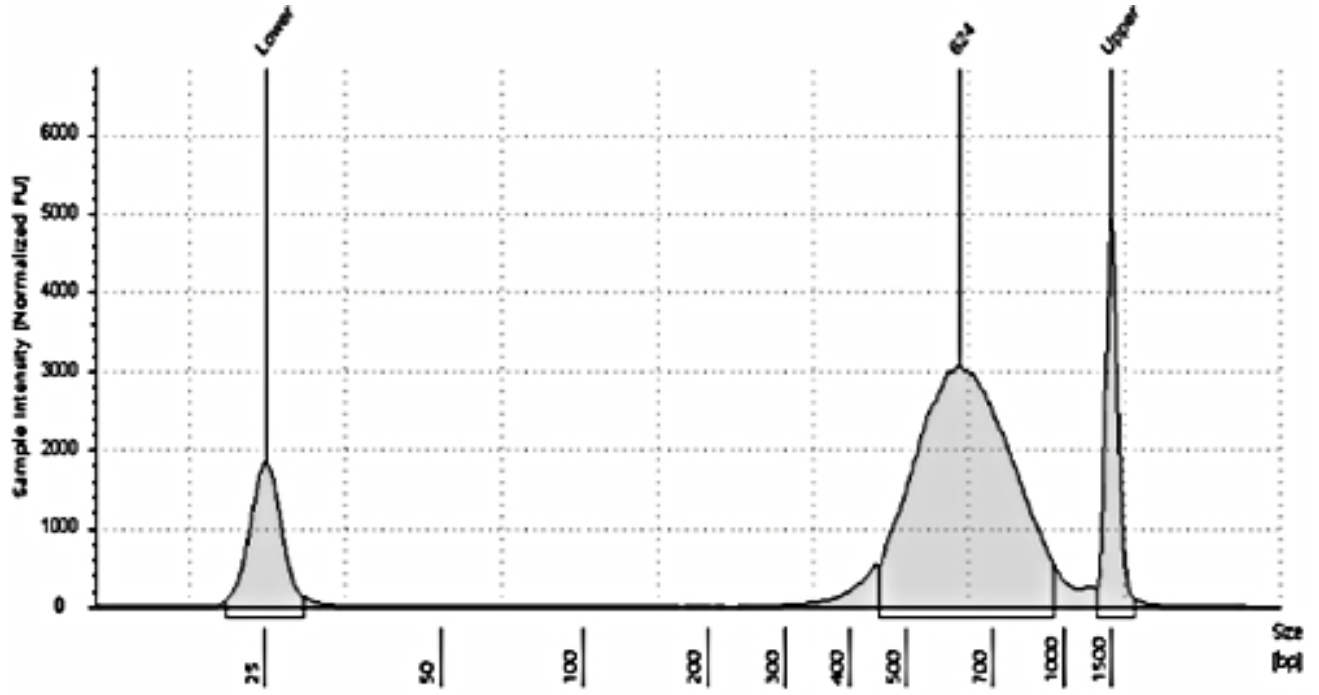

Figure 7. *qDNA. Aspergillus uessalvadorensis MACROGEN INC. 2024.*

In Figure 7. It is observed from the sample sent from El Salvador of the LightCycle qPCR, 624 bp were obtained at a concentration of 103.24 nM and 41.87 ng/ul with the TruSeq Nano DNA (350_META) library, indicating that the test goes to the next stage, which is the construction with 32 library kits ranging from 5 to 10 nM on the NovaSeq platform. It refers to quality and indicates a DNA preparation that has been treated and analyzed that meets certain standards of purity and quality, which is essential for applications in molecular biology, such as DNA sequencing or PCR. This involves the isolation of high-quality DNA using methods such as spectrophotometry or gel electrophoresis. Indicates that at high concentrations: DNA samples >10 ng/µL are ideal for sequencing, as they ensure that there is enough genetic material to generate accurate readings. On the other hand, at low concentrations: they may require additional concentration or repeat extraction to ensure sufficient DNA for analysis. To sequence a fungus a number of base pairs needed to sequence fungi can vary depending on the objective of the study and the type of sequencing being used. To get proper coverage of a fungus's entire genome, millions of base pairs usually need to be sequenced. The exact amount depends on the size of the fungus's genome in question. For example, fungal genomes can range from a few megabases to more than 100 Mb megabases. If you want to sequence specific regions or certain genes, the number of base pairs required will be much lower. This could be on the order of thousands or millions of base pairs, depending on the number of regions of interest. In other words, it is quantified DNA. [21-37].

6) cDNA library

33 bookcase kits were used**.** It refers to evaluating the quality and representativeness of the cDNA library prepared for sequencing. This technique is often used to create a complementary DNA copy of mRNA from a specific organism. It also refers to the visualization of the quantity and/or quality of double-stranded DNA extracted from a sample for subsequent analysis, such as sequencing. If the fragments are short: it is an indication of RNA degradation or inefficient library preparation. If fragments are long: indicates problems in RNA fragmentation during preparation. That is, it refers to complementary DNA. This type of DNA is generated from messenger RNA (mRNA) through a process called reverse transcription. cDNA is used to create a sequencing library. cDNA fragments are prepared by adding adapters to their ends. These adapters allow fragments to bind to the surface of the cell stream and amplify during the sequencing process.

7) dsDNA

Refers to double-stranded DNA, if it has a high concentration: A high value on the graph indicates a good amount of double-stranded DNA, which is ideal for sequencing. For example, concentrations above 10 ng/µL are generally considered suitable for most sequencing techniques. If it is low concentration: indicates problems with DNA extraction and may require additional concentrations. That is, double-stranded DNA is denatured into single-stranded DNA to allow the reading of nucleotide sequences. During sequencing, these DNA fragments are amplified and read to determine the correct nucleotide sequence. In Macrogen it is the FASTQ Files that contain the nucleotide sequences read directly from the sequencing machine, accompanied by quality information. Metagenome Shotgun Sequencing Report 2024.09 (SMSR):

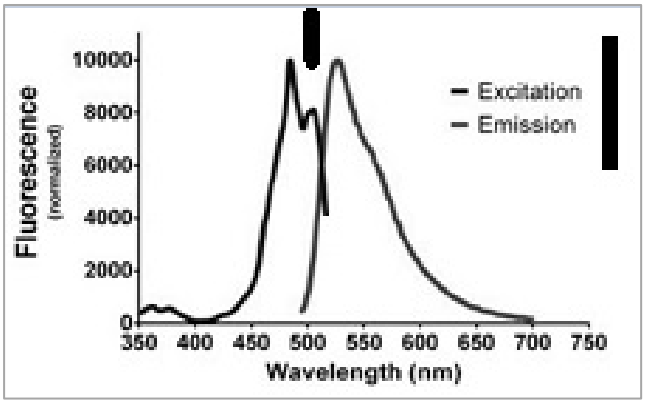

Figure 8. *dsDNA extraction.*

1) Total bases of a genome: bp. a value of 11,705,895,990 bp is reported. This value indicates in the context of DNA refers to the total amount of nucleotides (or nitrogenous bases) present in a DNA sample. In this case, it could indicate the total amount of bases in picomoles, or some other quantitative measure, that are present in the sample being tested. That is, count of how many guanines, cytokines and adenine and thymines in the sample. That is, 5,735,889,035.1 were GCs that contain this number of nucleotides that contain the GC nitrogenous base and 5,852,947,995 were AT, that is, there is this number of nucleotides that contains the AT nitrogenous base. In percentage, the total bases sequenced in Gb expressed as a percentage was 11.7, that is, the total amount of nucleotides or nitrogenous bases present in the DNA sample. In its effect, 11.7 the total amount of picomoles present in the analyzed sample.
2) -Total readings: Value. 77,522,490. This could indicate that a total of 77,522 reads have been recorded. 77,522,490 reads are produced and the total read bases are 11.7 Gbp. In other words, 66% of DNA reads is the proportion of sequences that were successfully read from the DNA fragments that have been sequenced and then assembled or aligned to reconstruct the entire sequence. This percentage also indicates that it has been sequenced correctly and has generated quality data and the remaining 34% could correspond to failed or low quality readings or that could not be aligned or assembled correctly.
3) GC/AT Content: has a GC % 49.7 and AT % 50.30. It

indicates that there is 49.7% guanine-cytokine and 50.30% adenine-thymine.

4) GC Content: has a value (%) of 49.7% suggests a considerable balance between GC and AT base pairs, which can influence the stability and properties of the DNA sequence. GC percentage: 50%. It indicates that 50% of the bases in the DNA sequence are guanine or cytosine, while the remaining 50% are adenine or thymine. This percentage is a measure of the balance between these two types of base pairs in a sequence.
5) AT Content: The value "55.0 AT %" refers to the percentage of adenine-thymine (AT) base pair content in a DNA sequence. That is, the composition of DNA, the bases adenine (A) and thymine (T), pair with each other by two hydrogen bonds. The AT content in a DNA sequence refers to the ratio of adenine and thymine bases compared to the guanine and cytosine bases. An AT percentage of 50.0 AT %" means that 50% of the bases in the DNA sequence are adenine or thymine, while the remaining 50% corresponds to guanine or in In general, a percentage of 50.0% AT in a DNA sequence indicates that most of the sequence is made up of adenine-thymine base pairs, with a balance in the base pairs.
6) The Q20 and Q30 metrics: their value is Q 20 95.1 and Q 30 88.3. A Q value of 20 of 95.1 indicates that it has a high score, which is good, because if it were low it would indicate low quality which did not happen.

Quality per cycle:

Figure 9. *Macrogen Cycle Data Quality.*

In Figure 9, reading 1 reading 2 indicates the degree of identification in each of these cycles. It is commonly represented on a graph where the X-axis shows the cycles at positions in the sequence, and the Y-axis shows a measure of quality, often in the form of a quality score such as the Q-value. It is a quality analysis performed by FASTQC for raw readings of the Aspergillus genome. At the top it refers to quality. In the lower part it is observed in the direction 5" to 3 " and vice versa in the opposite direction. X-axis is the bases and the Y-axis is the value of Q30. The colors represent quality, so green indicates high quality, yellow indicates intermediate quality and low quality pink. A high quality value per cycle suggests that the sequencing is accurate and that the bases in that specific cycle are correctly identified. A low value could indicate problems with reading at that point, such as sequencing errors or problems with the sample.

1) Raw data. They report a value. 38,761,245 n is the number of reads or fragments of DNA that were generated during the experiment.
2) Adapter quality and trimming. (Quality and adapter trim.). They report a value of 32,535,420 that unwanted adapters were removed.
3) Elimination of contaminants. (Contaminant removal). They report a value of 30,961,740. It refers to 30,961,740 as a high value that was identified as contaminants and immediately eliminated during the sequencing process. These contaminants can include unwanted sequences, such as DNA from bacteria, viruses, or even unwanted human DNA fragments if you are working with a sample from another organism. Contaminant removal is a crucial step in analyzing sequencing data to ensure that the results are accurate and reflect only the sequences of interest. This helps to improve the quality and reliability of the final data used in subsequent studies.

4) Quality control of previous reading. (QC passed read). They report a value of 79.88 indicating that it was accepted as good performance of the process.

Taxonomic analysis: - Krona taxonomy:

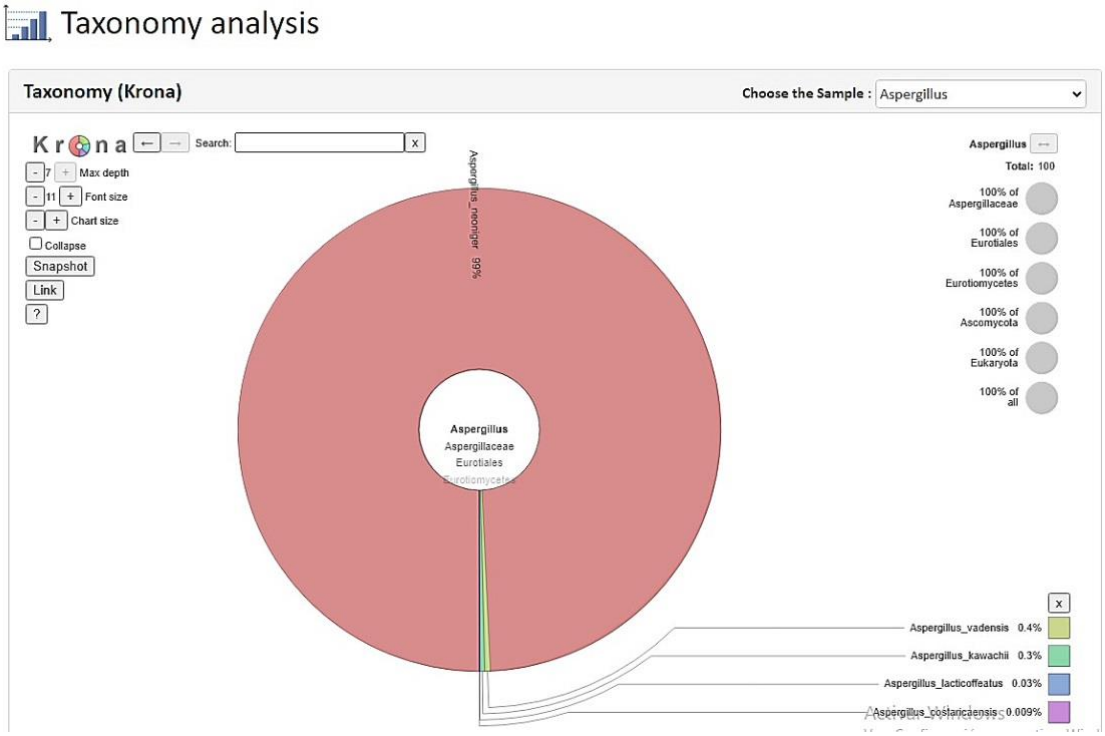

Figure 10. *Taxonomy Krona. Aspergillus sp. MACROGEN.*

In Figure 10 Analysis: From the sequencing report, there are varieties similar to the sample, 99% *of Aspergillus neoniger. Aspergillus vadensis 0.4%, Aspergillus kawachii 0.3%, Aspergillus lacticoffeatus 0.03% and Aspergillus costaricaensis 0.009%. Molecular bioinformatics analysis showed that it is another similar species not discovered and described in EL SALVADOR compared to other species. It is clarified that the report is circular DNA.*

Molecular analysis. *Aspergillus* from the sample sent from El Salvador. For molecular analysis, the following programs were used: BLAST Basic Local Alignment Search Tool, MEGA 11 Molecular Evolutionary Genetics Analysis, CLUSTAL OMEGA Multiple Sequence Alignment program, Nucleotid BLAST program, GENEBANK Genetic Sequence database and others no less important. By analysis of molecular studies with the Clustal Omega Multiple Sequence Alignment (MSA) program. It was determined that the strain is of the genus Aspergillus but its species is similar but not the same as that reported by DNA type.

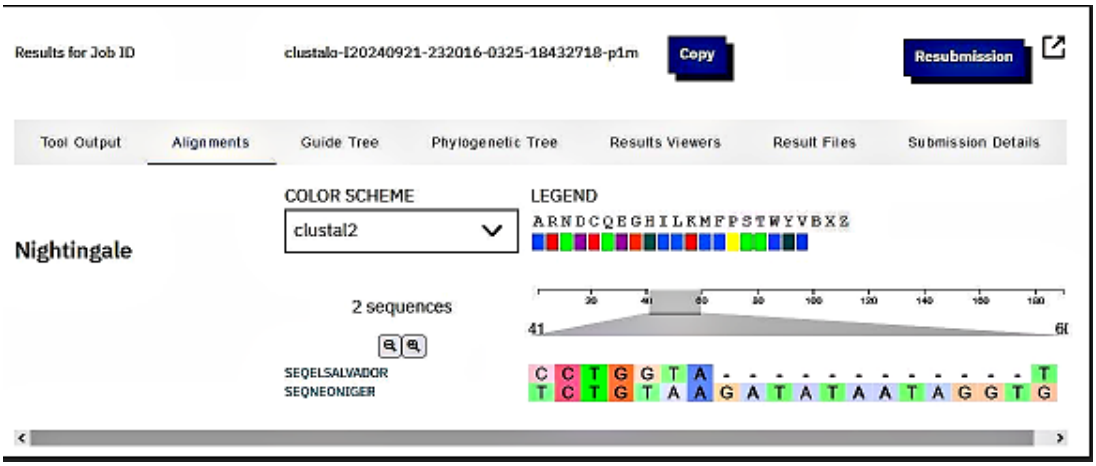

Figure 11. *Genome comparison of the species of El Salvador and that reported by DNA type according to the base sequence.*

Figure 11 shows that a segment of DNA sequences from El Salvador 1 with the sequence from the species Neoniger 2 is not the same. The similarity between the Cytokine and Guanine bases can be seen in the graph, it is not a higher percentage that varies in range between 40 and 60 that of El Salvador in relation to the neoniger strain that is between 41 and 60 nucleotides of Pb. That is, CC, GG and AA coincide in 0.15% with that of the neoniger species reported by DNA type. White spaces are gaps that do not match the neoniger species.

Type of relationship between the species uessalvadorensis, neoniger and niger.

Figure 12. *Relationship between the species uessalvadorensis, neoniger and niger.*

In Figure 12 it can be seen that the three species do not coincide in the sequence, but only in 8 aligned cases that are marked with an asterisk they coincide in the sequence of GGG, CCC, AAA, TTT preserving a relationship but the rest of the bases are different. So the three species are not the same in the guidelines, so they may have had mutations or non-conserved regions.

Aspergillus Open Reading Frame Sample from El Salvador

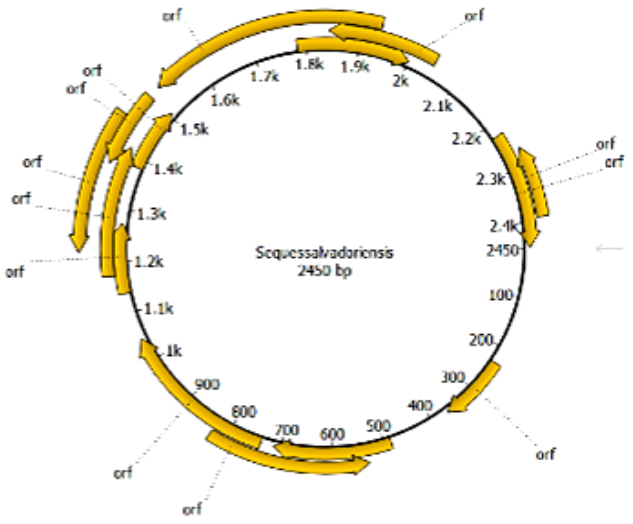

Figure 13. *ORF plot of the sequence Sequessalvadorensis of 2450 bp.*

In Figure 13 is a circular ORF (Open Reading Frame) graph involves interpreting the graphical representation of the reading frames in a biological context, usually in genome studies. Circular Structure: The graph is usually circular as well as the results are circular DNA, representing the DNA sequence. The points or segments in the circle correspond to different regions of the DNA. Reading Frames: Look for open reading frames, which are marked and presented as lines or blocks. Each ORF represents a possible coding sequence for a protein. Direction: Observe the direction of the ORFs. They can be in the direct chain (5' to 3') or in the complementary chain (3' to 5'). This indicates how they are transcribed and translated. Size: ORFs of different lengths can indicate different coding potentials. Generally, a longer ORF is more likely to encode a functional protein. In a pie ORF chart, "1.7k" usually refers to the length of an open reading frame (ORF) measuring 1,700 base pairs (bp) in length. The "k" stands for "kilo," which in this case means a thousand. This implies that the ORF in question could encode a protein, and its length is relevant to understanding its potential function. An ORF of 1.7k is relatively long and could be indicative of a gene encoding a protein of significant size. In a pie ORF chart, arrows usually have specific meanings related to the genetic information represented. According to Transcription Direction: Arrows indicate the direction in which DNA is transcribed to RNA. Normally, they point from 5' to 3', which is the direction in which RNA is synthesized. Gene orientation: They can show whether the gene is in the sense chain or in the chain complementary (antisense). An outward arrow in the circle indicates a gene in the direct strand, while an inward arrow may represent a gene in the complementary strand. Promoter or Terminator Signals: Some arrows can point to the location of promoters or terminators, which are regulatory elements that control gene transcription. Relationship between ORF: In more complex graphs, arrows can connect different ORFs or genes, indicating functional or regulatory relationships between them. On a pie ORF chart, arrows pointing to to the left or right usually indicate the direction of

transcription of the open reading frames (ORF). Here's more about what it means: Right arrows: They indicate that the corresponding ORF is located on the DNA strand in the 5' to 3' direction, which means that it is being transcribed from that strand This is considered the sense or direct string. Arrows to the left: Indicate that the ORF is in the complementary chain, transcribed in the direction 3' to 5'. This chain is known as antisense. In short, the direction of the arrows provides information about how RNA is synthesized from DNA and the orientation of genes in the genome is oriented in the opposite direction to other genes in the same region of the genome.

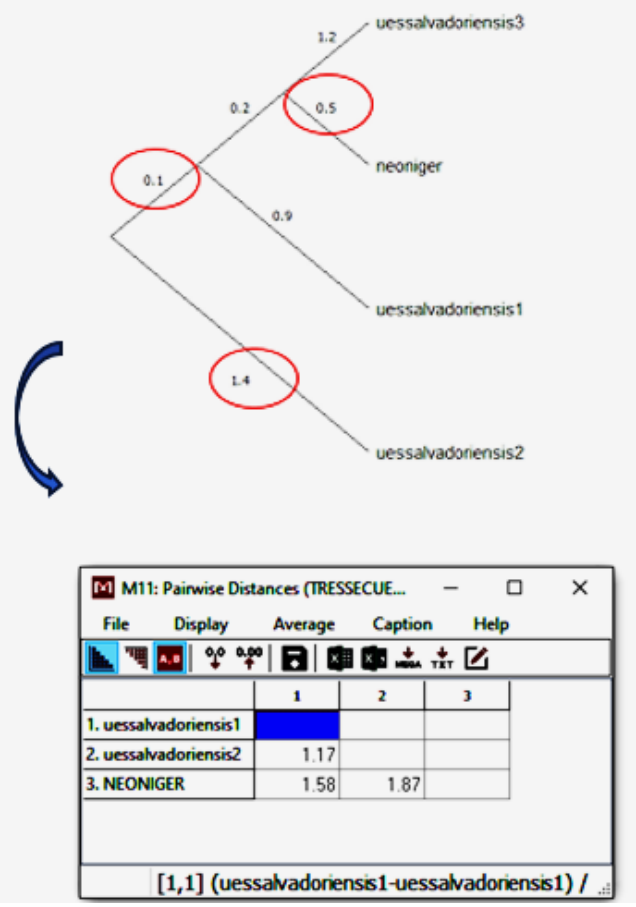

Figure 14. *Aspergillus evolution phylogeny.*

In Figure 14 Evolutionary analyses were performed in MEGA11. It indicates a DNA or RNA sequence from different samples, probably from a genetic analysis. Each line appears to represent a different sequence, with a species or sample name followed by the nucleotide sequence and its length. uessalvadoriensis2 and uessalvadoriensis1 they seem to refer to samples of a species that could be endemic to El Salvador, given the name among varieties identified as neoniger could refer to another species or sample, possibly an organism that has been studied in the same context. The numbers at the end of each line could indicate the length of the sequence in bases. The numbers (1.17, 1.58, 1.87): These could be values that represent some measure, such as genetic distance, divergence between sequences, or some other type of comparison (such as substitution rates or diversity measures). In this context, they could indicate the similarity or genetic difference between the samples. Numbers such as 1.17, 1.58, and 1.87, in the context of a genetic analysis, are usually interpreted as measurements of genetic distance between the DNA sequences of the samples. Genetic distance: These numbers usually represent the amount of nucleotide changes or differences between sequences. A lower number suggests greater genetic similarity, while a higher number indicates that the sequences are more different. Scale: The scale of distances can vary depending on the method used to calculate them (e.g., Kimura distance, Jukes-Cantor distance, etc.).

It is important to know which method was used to correctly interpret the values. Comparisons: If the sequence uessalvadoriensis1 and uessalvadoriensis2 are 1.17 apart, this could indicate that they are quite similar genetically. On the other hand, when comparing uessalvadoriensis1 with *neoniger* there is a distance of 1.58, which suggests that there is a notable difference between them. A distance of 1.87 between uessalvadorensis2 and *neoniger* indicates an even greater difference, which could mean that these two species are more distant evolutionarily. Evolutionary interpretation: These distances can provide information about the evolutionary history of species, helping to build phylogenetic trees that show the relationships between species. In the context of phylogeny, a "position" of 58 usually refers to a specific location on a phylogenetic tree, which is a graphical representation of the evolutionary relationships between different species or groups. According to the position number: It can indicate that a species is ranked 58th in the phylogenetic tree. This means that, when counting from the root of the tree to the leaves (the most modern species), that particular species is in the 58th position, indicating that there has been an evolutionary divergence. Branch length: In some phylogenetic trees, numbers may be related to branch length, indicating the amount of genetic changes that have occurred in that evolutionary line. Sequence identification: If you're analyzing a dataset, the number 58 could be a reference to a specific sequence, making it easier to identify in analysis. Comparative analysis: It can be used to facilitate comparison between species or groups in studies where they have been ordered according to their genetic characteristics. We have uessalvadorensis1 - uessalvadorensis2: The distance is 18.62, which indicates that there is a relatively small evolutionary difference between these two sequences. uessalvadorensis1 - neoniger2: The distance is 20.29, which also indicates a moderate evolutionary difference. uessalvadorensis2 - neoniger2: The distance is 32.89, suggesting a greater evolutionary difference compared to the previous pairs. Last row (neoniger): There seems to be an additional value that includes a comparison with neoniger. The distances indicate how the neoniger sequences are related to the other two: neoniger - uessalvado-

riensis1: 40.58, neoniger - uessalvadoriensis2: 42.65, neoniger - neoniger2: 50.41. General interpretation: Lower numbers indicate that sequences are more closely related evolutionarily, while higher numbers suggest greater divergence. This could be used to infer phylogenetic relationships, such as which species are most closely related to each other and how they have evolved over time. The farther away they are from the horizontal axis, the more they tell us that there are genetic differences between the others and that they have evolved. Of the proteins studied in *Aspergillus*, *Protein* A was found 594 times, which represents 24.24% of the total observations. Protein B: Not found in any observations, meaning its percentage is 0.00%.

4. Discussion

Diseases of the genus Aspergillus have been described in patients with low defenses, with pulmonary Aspergillosis being the most relevant. [1-6, 19-28, 41, 42, 47] PCR (Polymerase Chain Reaction) was invented by Karry Mullis in 1983. The use of PCR for the diagnosis of Aspergillus has been reported since the early 1990s. [19-27, 36-44, 48-55]. In 2006, the European Aspergillus PCR Initiative was established, in which it proposed standardized protocols for the generalized clinical evaluation of Aspergillus PCR in a rapid and timely manner in the diagnostic criteria of the disease. Another Mass spectrometry is based on obtaining ions from molecules in the gas phase; Once these ions are obtained, they are separated according to their mass and charge, and finally detected by a MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) mass spectrometer device. [54]. Modern sequencing techniques are second and third generation. The second generation produces a large number of short reads of 25-400 bp of DNA sequences. In this generation, the company Illumina Inc stands out, currently it is the company that offers quality services and strict control in its procedures is the vanguard of this century. The concept behind NGS technology is similar to Sanger's sequencing techniques. The original principle of Sanger's method is the use of deoxynucleotides as terminators of the DNA strand, where it prevents further replication. So it is divided into four reactions of separate sequences that contain the four standard deoxynucleotides that are: dATP, dGTP, dCTP, dTTP and DNA polymerase. They terminate the elogation of the strand, allowing for DNA fragmentation of various length sizes. [45]. Instead, Illumina's technology uses the synthesis sequencing method, allowing the parallel reading of millions of fragments by detecting the individual bases as they are incorporated into growing DNA strands. His method consists of sequential cycles of synthesis. During each cycle the nucleotides are identified by fluorophore excitation, creating an image of a fluorescently labeled reversible terminator as each dNTP is added, and then separated for input into the next base. At the end, a base-by-base sequencing is obtained with high-precision and high-quality data. Third generation sequencing (TGS) are the massive nucleic acid sequencing (TGS) techniques, which allow extensions up to 2.3 Mb and do not require preliminary amplification. [25] There is a Single Molecule, Real-time (SMRT) technology that allows the incorporation of readings of about 60,000 base pairs (bp), which offers an advantage of tracing structural variations or that present difficulties in the amplification of certain areas of the genome of a particular study. sequencing using nanopores Its disadvantage is that it has the limitation that a large amount of DNA is required to be able to sequence and obtain a reliable result. [5-21, 52]. In other studies it reports that the size of the genomes is varied among the various species of *Aspergillus spp*, these vary between 28.81 and 33.289 kb, their difference in size is from 323 to 4.479 kb. The number of genes varies between 9630 and 12074 [49] in our case it was 15000 bp. In short, after gDNA extraction in quality control, a qPCR is carried out in which a qPCR machine measures the intensity of the fluorescence emitted by the probe in each cycle. It is also known as quantitative real-time PCR, or qPCR, which is a standard method for quantifying gene expression levels in a sample. In our study, a fluorescent probe was used. Real-time quantitative PCR (qPCR) assays have poor sensitivity in the PCR assay itself, which uses techniques such as selecting genes with multiple copies per genome to determine the total amount of DNA analyzed. [26-30, 44] Other studies report that molecular biology-based methods have been used to characterize and differentiate the genus *Aspergillus*, such as the analysis of the sequences encoding the genes of the eukaryotic ribosomal RNAs 18S, 5.8S and 28 [32]. In our case, DNA extraction was used as a molecular typing method. The RAPD technique has been used in genetic determination studies, to distinguish between strains of a species, as well as in taxonomic and evolutionary analyses [16-24, 29]. *Aspergillus* is filamentous composed of chains of cells, called hyphae, the type of fungi opposed to yeast, which are composed of a single round cell. [13]. *Aspergillus* can cause multiple pathological processes. Among the pathogenicity factors of this fungus are: Due to the small size of its conidia, allows its aspiration, causing infection in the lung and paranasal sinuses; its ability to grow rapidly at 37 ℃, which makes it ideal for affecting humans; its ability to adhere to epithelial and possibly endothelial surfaces and its great tendency to invade blood vessels. To study a particular fungus, organic samples or studies of metabolites that are specific to fungi can be used to differentiate the different species of the genus Aspergillus. [17]. Illumina's sequencing technology, currently a sequencing by synthesis (SBS), is a next-generation sequencing (NGS) technology. Illumina's NGS technology uses a fundamentally different approach from the classic Sanger chain termination method. Illumina's latest SBS technology uses XLEAP-SBS chemistry. An image of a fluorescently labeled reversible terminator is created as each dNTP is added and then cut to allow for the addition of the next base. The combination of short insertions and longer reads increases the ability to fully characterize any genome. Illumina sequencers

have higher quality sequencing. XLEAP-SBS chemistry in the NextSeq 1000 and NextSeq 2000 systems. The NextSeq 1000 and NextSeq 2000 systems are carefully designed to allow you to get more information in your lab, with 14 settings and read lengths from 1 × 50 bp to 2 × 300 bp, makes 1.8 billion single-end reads per run to power the most data-intensive applications in your sequencer, these single-ended reads per run 100 million-1.8 billion Maximum read length 2 × 300 bps. It makes up to 384 unique dual combinations (UD) and 96 dual combinatorial combinations (CD). processes 1-500 ng of DNA for small genomes or 100-500 ng of DNA for large genomes. Target insertion size ~350 bp. Next generation sequencing (NGS) sequencing thousands of organisms in parallel, with the ability to combine many samples into a single sequencing and obtain high sequence coverage per sample, NGS-based metagenomic sequencing can detect very low abundance microbial community members who could overlooked or that are too expensive to identify using other methods. The Miseq system makes Maximum individual reads per execution 25 million and Maximum Read Length 2 × 300 bp. [25] In our study, this Illumina technique was used plus Metagenomic Sequencing Report of escopeta llamado Shotgun Metagenomic Sequencing Report as described above.

5. Conclusions

Studies to demonstrate a particular species relied on phenotypic and genotypic characterization for taxonomic identification of the study sample, in which both methods complemented each other. A new subspecies of fungus of the genus *Aspergillus* was found in the seeds of nacascol that are native to our country and that are found in the northern part of the country. Concluding that the taxonomy of the description will be: Domain: Eukaryota, Kingdom: Fungi, Division: Ascomycota, Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae, Genus: *Aspergillus*, Species: *Aspergillus uessalvadorensis*. What will be called in the future binomial scientific name: *Aspergillus uessalvadorensis* A. Vásquez 2006. From the genus *Aspergillus* and species derives the acronym ues which stands for University of El Salvador together with *salvadorensis* of the country El Salvador.

Abbreviations

DNA	Deoxyribonucleic Acid
UES	University of El Salvador
CENSALUD	Center for Health Research
FASTA	Format for Nucleotide Sequences
MACROGEN	Macroscopic Phenotype of Gene
MALDITOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
MERK	Merck Sharp and Dohme
NGS	Sanger's Sequencing Techniques
ORF	Open Reading Frame
PCR	The polymerase Chain Reaction
SMRT	Single Molecule, Real-time
SMSR	Shotgun Metagenome Sequencing Report
SBS	Sequencing by Synthesis
TGS	Third Generation Sequencing

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Collaborators

MACROGEN, Inc. Public Biotechnology Company. South Korea./ Yubin Hong/ Jaewon Sim.-Dra Vianney de Abrego/ Molecular Biology laboratory CENSALUD UES.-Lic Marvin Stanley Rodríguez. / Molecular Biology laboratory CENSALUD UES.-Dra Sandy Ruiz. /UBM. México.

Author Contributions

Antonio Vasquez Hidalgo is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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