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DNA Sequencing of *Aspergillus niger*: Unraveling Genomic Insights and Biotechnological Applications

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Abstract

The dominant causal agents of plant diseases are fungi. Diverse strategies are used by plant pathogenic fungi to colonize plants and cause disease in them. Plant pathogenic fungi play important role in agriculture. They infect the cereal crops especially in stored condition. The present study was conducted with the main purpose to screen the fungal contamination on *Zea mays* seeds. *Zea mays* seeds were surface sterilised with 0.2% aqueous sodium hypochlorite and arranged on blotters to screen fungal contamination by standard blotting method. The fungal isolates were isolated from the collected seeds after 7 days. Identification of fungi was done by direct microscopy and molecular techniques such as DNA sequencing. Results revealed that *Aspergillus niger* was the most common isolate from *Zea mays.* Identification of fungi by molecular method using Polymerase Chain Reaction (PCR) helped in the confirmation of fungal species with precision and in the least possible time. In conclusion, the isolated species of fungi were identified morphologically as *Aspergillus niger*. The fungal samples were then identified through molecular techniques by DNA sequencing were identified as *Aspergillus niger.*

Keywords: Plant diseases, *Zea mays*, Standard blotting method, *Aspergillus niger*, DNA sequencing

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INTRODUCTION

The dominant causal agents of plant diseases are fungi. Diverse strategies are used by plant pathogenic fungi to colonize plants and cause disease in them. Some fungi kill the plant hosts and feed on dead plant tissue (necrotrophs), while the rest pathogenic fungi colonize the living tissue of plants (biotrophs).[1] Plant pathogenic fungi play important role in agriculture.[2] They cause about 85% of plant diseases[3], and severe infection in crop plants could result in crop yield reduction, increased production cost, fruit quality reduction, and even loss of life to farmers. Further, in some cases, contamination of fungi in grain products could predispose consumers to toxins inimical to health.[2]

Zea mays is the scientific name for maize, which is commonly known as corn. It is a cereal grain and one of the most widely cultivated and important staple crops worldwide. *Zea mays* belongs to the grass family (Poaceae) and is native to the America, where it has been cultivated for thousands of years. Maize is a highly versatile crop with numerous applications. It is primarily grown for its edible kernels, which are used as a food source for humans and livestock. Maize can be consumed fresh, cooked, or processed into various food products such as cornmeal, corn flour, corn oil, and corn syrup. It is a staple food in many regions, particularly in parts of Africa, America, and Asia. Apart from its use as a food crop, maize has several other industrial applications. It is utilized in the production of biofuels, animal feed, starch, and various types of bioplastics. Additionally, maize is an essential component in the production of many food and non-food products, including ethanol, sweeteners, oils, pharmaceuticals, and textiles. In agriculture, maize is grown in a wide range of climates and soil types, making it adaptable to various agricultural systems. It is a C4 plant, which means it is highly efficient at converting sunlight into biomass and is well-suited for warm climates with abundant sunlight. Maize cultivation practices can vary depending on the intended use of the crop, with different methods employed for grain production, silage production, or as fodder for livestock. Overall, *Zea mays* (maize) is a versatile and globally significant crop that plays a vital role in food security, nutrition, and various industries worldwide.[12, 13, 14]

Aspergillus niger is the fungi which belongs to genus *Aspergillus* and it is classified within the nigri section. [4] *A. niger* is cosmopolitan in distribution and is a common contaminant in soil, decaying plant material, compost and litter. It can withstand icy and marine environments but it prefers warm and dry environments.[5] *A. niger* is an opportunistic pathogen, its spore can aerosolized and have the capacity to be sublimated in bronchioles of the human respiratory tract.[6] Black mold infections in certain legumes, fruits, and vegetables such as peanuts, grapes, and onions are caused by *Aspergillus niger* and it is a common food contaminant.[7] *Aspergillus niger* can have detrimental effects on *Zea mays* (maize) under certain conditions. Here are a few ways in which *Aspergillus niger* can affect *Zea mays*:

- Post-Harvest Storage Losses: *Aspergillus niger* is a common post-harvest pathogen that can cause significant losses in stored maize. The fungus can infect maize kernels that are damaged or exposed to high humidity levels during storage. It produces enzymes that degrade the kernel's protective tissues, allowing for fungal invasion and colonization. *Aspergillus niger* can produce mycotoxins such as ochratoxin A, which can contaminate maize and pose health risks to humans and animals if consumed.
- *Aspergillus* Ear Rot: *Aspergillus niger* can cause ear rot in *Zea mays*. Ear rot is a condition where the fungus infects the maize ear, leading to rotting, discoloration, and decay. It typically occurs in maize ears that are damaged or stressed due to insect feeding, drought, or mechanical injury. *Aspergillus niger* infection can result in reduced grain quality, weight loss, and increased susceptibility to mycotoxin contamination.
- Aflatoxin Contamination: Although *Aspergillus niger* is not a primary producer of aflatoxins, it can facilitate the growth of aflatoxin-producing *Aspergillus* species, such as *Aspergillus flavus*, in

maize. Aflatoxins are highly toxic mycotoxins that can contaminate maize and pose significant health risks when consumed by humans or animals. *Aspergillus niger* can create favorable conditions for aflatoxin-producing fungi to thrive, exacerbating the risk of aflatoxin contamination in maize.

- Degradation of Maize Stover: *Aspergillus niger* is known for its ability to degrade plant material, including maize stover (the aboveground residues of maize plants after harvest). It produces a range of enzymes, such as cellulases and hemicellulases, that enable the breakdown of complex plant cell wall components. While this can be beneficial for the decomposition of crop residues, excessive *Aspergillus niger* activity may lead to the loss of organic matter and nutrient recycling in the soil.

It is important to note that the impact of *Aspergillus niger* on *Zea mays* can vary depending on environmental conditions, cultural practices, and the susceptibility of maize cultivars. Proper storage practices, including maintaining optimal moisture levels and temperature, can help minimize *Aspergillus niger*-related storage losses. Furthermore, good agricultural practices, such as timely harvesting, proper crop rotation, and disease management strategies, can reduce the incidence and severity of *Aspergillus*-related diseases in maize crops.

In the present study *Aspergillus niger* was isolated from *Zea mays* seeds collected from local markets and identification of *Aspergillus niger* was done by colony morphology, direct microscopy and molecular techniques such as DNA sequencing. Culture characteristics, direct microscopy and histopathology have been the base for identification of fungal infection from a long time. Every fungal species has the unique characteristics. Therefore, every single description of a fungal species is also distinctive. The morphological, physiological, ecological, and molecular diversity in fungi means that descriptions and characterization differ from one taxonomic group to another. But sometimes identification of fungi by colony characteristics and direct microscopy does not assure the correct species as spores of fungi in same genus looks alike and also some fungi produce different types of colonies. However, to overcome this, DNA sequencing of fungi is done to identify fungi at species level. DNA sequencing of *Aspergillus niger* is important for understanding its genomic structure, studying its pathogenicity and virulence, exploring secondary metabolism for biotechnological applications, improving industrial strains, monitoring contamination, and conducting comparative genomics studies. This knowledge has broad implications for human health, biotechnology, and industrial processes involving *A. niger.*

MATERIALS AND METHODS

Reagents used

- 0.2% aqueous sodium hypochlorite
- Insta Gene™ Matrix Genomic DNA isolation kit (Catalog # 732-6030)
- 1% agarose gels
- Ethidium bromide
- Distilled water

Sample collection

Random *Zea mays* seeds were collected from local markets.

Sample preparation

Zea mays seeds were surface sterilised with 0.2% aqueous sodium hypochlorite solution and 25 seeds per plate were arranged on the wet blotter by standard blotting method. Distilled water was added to maintain moisture content and the plates were incubated at 25 ± 2°C for 48 hours. After 48 hours fungus was isolated, subcultured and identified based on morphology and with the help of

direct microscopy as described by Barnet and Hunter,[8] Nelson et al.,[9] Pitt and Hocking,[10] Leslie and Summerall.[11] To validate the species, molecular techniques such as DNA sequencing was used.

Fig 1: *Aspergillus niger* on *Zea mays*

Fig 2: Sub-culture of *Aspergillus niger*

Molecular identification of fungal isolate

Extraction of DNA

Genomic DNA was isolated by using the Insta Gene™ Matrix Genomic DNA isolation kit (Catalog # 732-6030).[15]

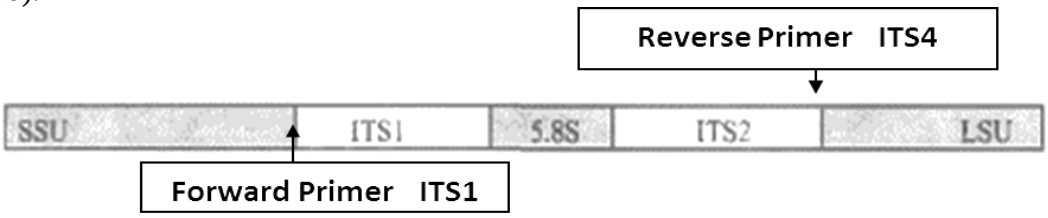

Fig 3: Ribosomal Gene organization and Target region amplified

Table 1: Primer details- Ribosomal RNA ITS Region Universal primers

ITS Primer for Fungi	**Sequence Details**	**Amplicon size (bp)**
Forward PrimerITS1	GGAAGTAAAAGTCGTAACAAGG	**620bp**
Reverse Primer ITS4	TCCTCCGCTTATTGATATGC	**620bp**

Polymerase Chain Reaction

Target gene fragment was amplified using Thermo Scientific Veriti Thermal Cycler PCR Protocol[16]: DNA fragments are amplified using 1 µL of template DNA in 10 µL of total PCR reaction mixture using ITS1F/ITS4Rprimers (50 pmol) and 30 amplification cycles with following program:

Table 2: Protocols of PCR

Initial denaturation	95℃ for 5 mins
Denaturation	95℃ for 1mins

Annealing	55℃ for 30 sec
Extension	72℃ for 1mins
Final Extension	72℃ for 7 mins

Purification of PCR products

Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).

Sequencing [17]

The PCR product was sequenced using the 1492R ITS1F/ITS4R primers. Sequencing reactions were performed using a ABI PRISM® Big Dye TM Terminator Cycle Sequencing Kits with Ampli Taq® DNA polymerase (FS enzyme) (Applied Biosystems). Single-pass sequencing was performed on each template using18S rRNA gene universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Bio systems).

RESULTS

The fungi were isolated from *Zea mays* seeds and identified as *Aspergillus niger* by microscopy and DNA sequencing.

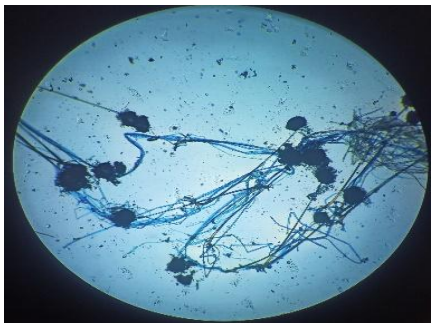

Fig 4: *Aspergillus niger* under stereoscopic binocular microscope (magnification - 400x)

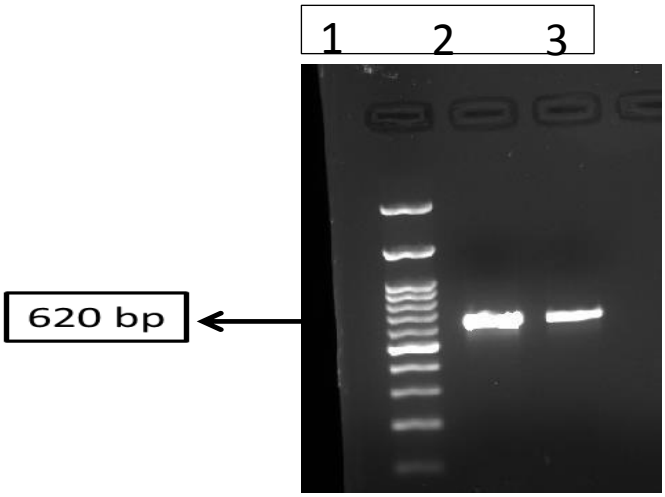

Fig 5: PCR Amplicon of ITS 1-4 :-Lane 1-Molecular size ladder, lane 2 – AN(5µl), lane 3 – AN (2µl)

The DNA sequence of *Aspergillus niger* is as follows

Forward Sequence

>0323_304_001_PCR_SLS_03_02_FORWARD_A02.ab1
TGGGAAGGGGGCCCTCCCGCAGCTTTGAAGTTTGGCCCCCCCCAAATGAATGTCCCCAC
GGATCGTTTGGGATTTTCCCTATTTTCGCGGATCTTCTTTTTTTTTAATTTCCGCCCACATC
CTTTTATTCATGGCGCCAAAAGCAAGAGACTGAATACCGCTATCATCTCCCTGTTTTCAA
AATTGTAAATAAGGGAGGTCGACAACGGGCCCAATGAACTCTTGCTGCTACTGGCGAAG
GTAGACAAAAACACGTTCTCTCCATACTTTTTCACGGAGAGGGGTCCGGCTGCATTTCTT
CGGAGGACTGTTCGGGGAGGCATGCAAAGGCAGGAGAGGACCCCCCGGCCTCGAATCG
AAAACAAGCCAACCGAAAAATTGCGAATGGAAATTCAAATTTCCCTTCTTGACCCTCGA
AGAGGCCACATACTAAGAGGGGAGGTGCGGAGGCCCCCCCCCCAACTCCCTGAAAAAT
ATGAGACCCTTAAAAACTTAACCTTGGATAAAAAAAAACCTGCAAGATATTCTTTTCAAAA
TATCTCTCGGAGGGGGGGGGCCAATCTGGGGGGGGAAATCCCCCACGGAACCAGGTGC
ACAAAAAGCG

Reverse sequence
>0323_304_002_PCR_SLS_03_02_REVERSE_A10.ab1
TAGCGGGAGGTCTGTACGATCGACACAAATTTTATCGAGAGATGGAAACGACTTCGGAG
GGAACATAACAGCTCCTCCAGTTGTCCAAAAACAAAATTTTCCGTCTGGCTCTAGATTCT
GATGAGCATTCTATTTATCATGTTTATCACTGAGATGAACACCTCGAGACCCACTGGGAC
CACTGGGTTCAGATAGGATTATTTGCCGTAACATGCAACGGATGGTGAGATATGTCAATG
AAGTCATTGGGGAAAATGATTGCTTTCTTTTTTCAATGCTTTTAACCTGATTAATCTCGTA
GATGGGGGTTTAGGGCCATACCCTGATTGATTAATTAAGGCTATGTTGTGGCAAATACAA
AAAATATGGGTAGACTAAGTGAGGCGCTGTCCTTCGCGCCGAAGGGGGCCGCCCCAAG
GCGGGAGGGGGGCCCCCCCCCCTCCCTTAAAAATGCGGGGTGGCCCAACTTCCAAGGG
AAAAAAAATGGGGGGGGAGTTTAAAAAGTAATTCCGTTGTCGTTGGCTTTTTGGAGGGG
GTCGGTAAGGACTTGAATATTTTTTCGTAGAAACTCCCGGGTTGTCACTCATAAGAACAA
AATCGCCCAAAATCCCGCCTCCCGCCTCTGGGCTTCTACTTAAGTATCGCCA

>Contiguous sequence
TGGCGATACTTAAGTAGAAGCCCAGAGGCGGGAGGCGGGATTTTGGGCGATTTTGTTCT
TATGAGTGACAACCCGGGAGTTTCTACGAAAAAATATTCAAGTCCTTACCGACCCCCTCC
AAAAAGCCAACGACAACGGAATTACTTTTTAAACTCCCCCCCCATTTTTTTTCCCTKGGA
AGTKGGGCCMYCCCGCAKYTTTAAGGGAGGGRGKKKGGCCCCCCTCCCGMMWTGGG
RMKGYCCCCTWCGGATCGYKWRGGAYWKYSCCTMWYTTMGYSKATCYTCATWTTTTT
TKWATTTSCSMCMACATASCYTTWATTMATGGCRMYMARRGYAWGRSMCTRAAYMCCS
MTMTACGAGATYWMYCWGKTTWMAARCATTGTAAATAARGRARGTCRATCAWYKKSC
CCAATGAMYTCATTGACWKMTACTSRCSATMSGTWGACAAWRWYACGTKCWMWYMA
TMCTWTYTSAMSGMSAGKGGTCCGGCWGYRKKTCTTCGGAGGACTGTTCAKSKSAGKS
ATGCAAAGGCATGRTARATRGAMYSCYCGRYCTMGAATCKARARCMAGCCAACSGAAA
ATWTTGYKWWTGGACAAYTSRARKWKCYSTTMTKKWCCCTCCGAAGTMGKTTCCATC
TMTMSATAARATTKGKGWSGWKCGKACRGMCCYCCCSCYAACTCCCTGAAAAATATGA
GACCCTTAAAAACTTAACCTTGGATAAAAAAAAACCTGCAAGATATTCTTTTCAAAATATC
TCTCGGAGGGGGGGGGCCAATCTGGGGGGGGAAATCCCCCACGGAACCAGGTGCACA
AAAAGCG

DISCUSSION

The advent of DNA sequencing technologies has revolutionized our understanding of *Aspergillus niger*, a filamentous fungus with immense biotechnological potential. Through the application of various sequencing platforms, including Next-Generation Sequencing (NGS) technologies such as Illumina, Pacific Biosciences (PacBio), and Oxford Nanopore sequencing, researchers have gained valuable insights into the genomic structure, gene expression, secondary metabolism, carbohydrate

utilization, and genetic engineering approaches of *A. niger*. The genomic studies on *A. niger* have provided significant advancements in our knowledge of its genome organization, including the assembly and annotation of its genome, the identification of gene content and organization, as well as the presence of repetitive elements and transposable elements. These findings have shed light on the evolutionary history and genetic diversity within *A. niger* populations. Secondary metabolism in *A. niger*, a major area of interest, has been extensively studied using DNA sequencing. The identification and characterization of polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), and other secondary metabolite gene clusters have facilitated the discovery of novel natural products with potential applications in the pharmaceutical industry. The elucidation of *A. niger*'s carbohydrate utilization capabilities has greatly aided in understanding its enzymatic machinery for degrading complex polysaccharides. This knowledge has opened avenues for exploiting *A. niger* in various biotechnological applications, including enzyme production for the biofuel and bioplastic industries, as well as lignocellulosic biomass conversion.

Genetic engineering techniques, particularly CRISPR-Cas9-based genome editing and synthetic biology approaches, have enabled the development of enhanced *A. niger* strains with improved traits for industrial production. These advancements hold promise for optimizing enzyme production, enhancing biofuel yields, and engineering *A. niger* as a versatile cell factory for the synthesis of valuable compounds. The biotechnological applications of *A. niger* DNA sequencing are far-reaching. Enzyme production, including amylases, cellulases, xylanases, pectinases, and lignocellulose-degrading enzymes, has been a significant area of focus. Additionally, *A. niger*'s potential in biofuel production and pharmaceutical applications, such as the production of therapeutic proteins and the synthesis of bioactive compounds, has been explored. Despite the substantial progress made in *A. niger* DNA sequencing, several challenges remain. Improving the quality of genome assemblies, enhancing functional annotation of genes, and expanding omics approaches to better understand the complex regulatory networks are areas that warrant further attention. [18,19]

CONCLUSION

In conclusion, DNA sequencing of *Aspergillus niger* has provided comprehensive insights into its genomic architecture, secondary metabolism, carbohydrate utilization, and genetic engineering potential. These findings have opened new avenues for biotechnological applications, allowing for enhanced enzyme production, biofuel production, and pharmaceutical development. Continued research efforts in *A. niger* DNA sequencing will undoubtedly drive further advancements, enabling the exploitation of this versatile fungus in a wide range of industrial sectors.

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