Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 5 (2023)

DNA Sequencing of *Aspergillus nomiae* Isolated from *Sorghum bicolor*: Insights via Polymerase Chain Reaction

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Abstract

This paper provides a thorough genetic analysis of *Aspergillus nomiae* strains isolated from *Sorghum bicolor* using PCR to study their genetic diversity and pathogenicity. Through a thorough literature review, we established the significance of *A. nomiae* in agricultural ecosystems and its potential impact on *Sorghum bicolor*, a vital cereal crop worldwide. The utilization of PCR allowed for targeted amplification of conserved regions within the fungal genome, enabling precise identification and differentiation of *A. nomiae* strains. Our results revealed a diverse genetic landscape among the isolated *A. nomiae* strains, indicating the existence of distinct genotypes with potential variations in virulence factors. Comparative analysis with existing genomic data sets highlighted unique genomic signatures, shedding light on potential adaptations and evolutionary pathways within *A. nomiae* populations. Furthermore, phylogenetic analysis elucidated the evolutionary relationships between the isolated strains and other *Aspergillus* species, providing valuable insights into the taxonomy and evolutionary history of *A. nomiae*. This research contributes to a deeper understanding of the genetic diversity within *A. nomiae* populations and underscores the importance of molecular techniques like PCR in studying fungal pathogens.

Keywords: DNA sequencing, *Aspergillus nomiae*, *Sorghum bicolor*, Polymerase Chain Reaction, Genetic diversity, Phylogenetic analysis, Agricultural pathogens.

Introduction

Aspergillus nomiae is a filamentous fungus belonging to the genus *Aspergillus*.[1] It is of significant interest in the fields of biotechnology and agriculture due to its diverse metabolic capabilities and its potential impact on agricultural ecosystems.[2] In biotechnology, *A. nomiae* has garnered attention for its ability to produce a range of secondary metabolites, including various enzymes and bioactive compounds. Some strains of *A. nomiae* are known to secrete enzymes with industrial applications, such as amylases, lipases, and proteases. These enzymes find utility in a wide array of processes, from food processing to biofuel production, highlighting the fungus's potential as a valuable biotechnological resource.[3] In agriculture, *A. nomiae* plays a pivotal role as a plant-pathogenic fungus.[4] It is commonly associated with *Sorghum bicolor*, a vital cereal crop worldwide. *A. nomiae* infections can lead to various plant diseases, potentially causing significant yield losses. Understanding the genetic diversity and virulence factors of *A. nomiae* strains is crucial for developing effective management strategies and breeding programs to enhance crop resilience against these pathogens. Moreover, *A. nomiae*'s ecological role in soil and plant microbiomes is an area of ongoing research. Its interactions with other microorganisms and its impact on nutrient cycling contribute to the complex dynamics of agricultural ecosystems. Overall, *Aspergillus* *nomiae* stands as a versatile and impactful organism, with implications ranging from biotechnological applications to agricultural sustainability. Its study holds promise for advancing both industrial processes and agricultural practices.

Sorghum bicolor, commonly known as sorghum, is a versatile and ancient cereal grain belonging to the grass family Poaceae. It is one of the most important staple crops worldwide, particularly in regions with arid and semi-arid climates. This hardy plant is native to Africa but has spread to various continents, adapting to diverse environments. *Sorghum* exhibits impressive genetic diversity, with thousands of varieties cultivated for different purposes, including food, fodder, and industrial applications. This drought-tolerant crop plays a crucial role in food security, especially in regions where water resources are limited. Its adaptability to harsh conditions makes it a resilient option for farmers facing unpredictable climates. Sorghum is a rich source of essential nutrients like carbohydrates, protein, fiber, and minerals. It is utilized in various culinary forms, from whole grains to flour, and is a primary ingredient in traditional dishes and beverages. Furthermore, sorghum holds promise in biofuel production due to its high biomass potential and efficient photosynthetic capabilities. Its unique traits make it an important focus of agricultural research, aiming to enhance its productivity and sustainability, thereby contributing to global food security and sustainable development.[8,9,10]

ITS (Internal Transcribed Spacer) primers are crucial in molecular biology for studying fungi. They target the ITS regions within the fungal rRNA gene cluster. These regions exhibit high variability between species, aiding in accurate fungal identification. ITS primers enable amplification of these specific DNA segments via PCR. The resulting sequences are used for taxonomic classification, biodiversity assessment, and ecological studies. Due to their conserved flanking regions, ITS primers are versatile and widely applicable across fungal taxa. Their significance lies in providing a standardized tool for fungal diversity research, facilitating insights into ecological roles, phylogenetic relationships, and aiding in environmental and medical studies involving fungi. DNA sequencing is crucial for unraveling genetic characteristics as it allows scientists to decipher the precise sequence of nucleotides within an individual’s DNA. This information serves as a blueprint for the genetic code, revealing the unique arrangement of genes responsible for traits, susceptibility to diseases, and overall biological functioning. By identifying variations or mutations in this sequence, researchers can pinpoint genetic predispositions, track hereditary conditions, and even develop personalized medical interventions. Moreover, DNA sequencing plays a pivotal role in fields like evolutionary biology, forensics, and agriculture, offering insights into the relationships between organisms, aiding in criminal investigations, and optimizing crop breeding programs. In essence, DNA sequencing is the cornerstone of modern genetics, providing a fundamental understanding of the genetic underpinnings that shape individuals and species.[5,6] The objectives of present study include: Genetic Characterization: Determine the genetic makeup and variations within *Aspergillus nomiae* strains isolated from *Sorghum bicolor*, Species Identification: Confirm the identity of the isolated *Aspergillus nomiae* and ensure it matches the known genetic sequences of this species, Phylogenetic Relationship: Investigate the evolutionary relationships between different strains of *Aspergillus nomiae* and possibly related species, Pathogenicity and Toxigenicity: Assess the potential pathogenicity and production of mycotoxins by the isolated strains, which is crucial for understanding their impact on *Sorghum bicolor*, Biodiversity and Ecological Insights: Contribute to the understanding of the diversity and ecological roles of *Aspergillus nomiae* within the context of *Sorghum bicolor*, Method Validation: Validate the use of Polymerase Chain Reaction as a reliable technique for DNA sequencing in this specific context. This research paper presents a comprehensive analysis of the genetic composition of *Aspergillus nomiae* strains isolated from *Sorghum bicolor*, a crucial staple crop.

Literature Review

The study from Moore G.G et al., provides valuable insights into the genetic makeup of the *A. nomius* strain and offers a basis for further research and understanding of this species. In their investigation, they found that the *A. nomius* type strain (NRRL 13137) possesses a genome size of around 36 megabases (Mb), a measurement consistent with other sequenced *Aspergillus*. Within the genome, there were an estimated 11,918 predicted genes, with approximately 72% of them having been annotated with Gene Ontology (GO) terms through the use of BLAST2GO.[11] In the research conducted by Kjærbølling et al., they provided comprehensive whole-genome sequences for 19 distinct species belonging to the *Aspergillus* section Flavi.[12] Similar to their study, in the present study DNA Sequencing of *Aspergillus nomiae* was done. Bader Alshehri et al., examined a sum of 135 isolates derived from cases of *Aspergillus* keratitis. They conducted sequence analyses of the internal transcribed spacer (ITS) region, employing nucleotide-nucleotide BLAST analysis. This was preceded by an initial identification of the isolates, which was based on assessments of conidial and colony morphology.[13] Similarly in the present study ITS primers were used to study the genome of *Aspergillus nomiae*. Tapani Yli-Mattila et al., investigated the phylogenetic relationships between aflatoxigenic and non-aflatoxigenic *Aspergillus* isolates. The researchers employed a polyphasic approach, integrating phylogenetic, sequence, and toxin analyses. They analyzed 40 *Aspergillus* section Flavi isolates gathered from eight different countries across the globe (USA, Philippines, Egypt, India, Australia, Indonesia, China, and Uganda). Through this method, the study identified the critical genomic characteristics that differentiate isolates capable of producing aflatoxins from those that do not.[14] Similarly, in the present study we studied the phylogenetic relationships between the closely related species of *Aspergillus nomiae*.

Materials and Methods

- Collection of Seeds Sample

Sorghum bicolor seeds were collected from Mandya, Karnataka

- Isolation of Fungal Sample by Standard Blotting Method

Sorghum bicolor seeds were surface sterilized with 0.2% sodium hypochlorite followed by rinsing in water and arranging on wet blotting paper in a sterilized petriplate. Drops of water were added twice a day to maintain moisture content for seven days. After seven days fungal sample was isolated from the seeds and identified as *Aspergillus* by colony characteristics and direct microscopy with the help of lactophenol cotton blue method.[7]

- DNA Sequencing

Genomic DNA extraction: Genomic DNA was isolated by using the Insta Gene TM Matrix Genomic DNA isolation kit (Catalog # 732-6030)

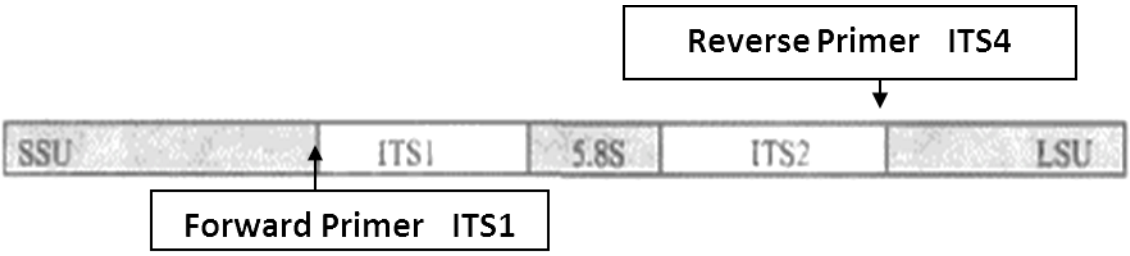

Fig 1: Ribosomal Gene organization and Target region amplified

Table 1: Primer Details: Ribosomal RNA ITS Region Universal primers

ITS Primer for Fungi	Sequence Details	Amplicon size (bp)
Forward Primer ITS1	GGAAGTAAAAGTCGTAACAA GG	**620 bp**
Reverse Primer ITS4	TCCTCCGCTTATTGATATGC	

Polymerase Chain Reaction: Target gene fragment was amplified using Thermo Scientific Veriti Thermal Cycler

PCR Protocol: 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: PCR Protocol

Initial denaturation	95°C for 5 mins
Denaturation	95°C for 1 mins
Annealing	55°C for 30 sec
Extension	72°C for 1 mins
Final Extension	72°C 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: 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

Forward Sequence

>0723_590_001_PCR_FL_FORWARD_B03.ab1

TCTGCTAGGTGACTGCGGAGGACATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCG
TGTTTACTGTAACCTTAGTTGCTTCGGCGGGCCCGCCTTTAAGGCCGCCGGGGGGCATCAGCCCCCG
GGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGC
AATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCG
ATAACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGT
ATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGT
CGTCCCCTCTTCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGT
ATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAAACAACCATTCTTT
CCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAAGCGGAGGAA

Reverse sequence

>0723_590_002_PCR_FL_REVERSE_B03.ab1

GAGGGCACCTACCTGATCCGAGGTCAACCTGGAAGAATGGTTGTTTTGCGTTCGGCAAGCGCCGGC
CGGGCCTACAGAGCGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGCGGTGCCGCCGCTGCCT
TTGGGGCCCGTCCCCCCCGAAGAGGGGACGACGACCCAACACACAAGCCGTGCTTGATGGGCAGC
AATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGAT
GATTCACGGAATTCTGCAATTCACACTAGTTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACC
AAGAGATCCATTGTTGAAAGTTTTAACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGAC
AGAGTTCGTGGTGTCTCCGGCGGGCGCGGGCCCGGGGGCTGATGCCCCCCGGCGGCCTTAAAGGC
GGGCCCGCCGAAGCAACTAAGGTTACAGTAAACACGGGTGGGAGGTTGGGCTCGCTAGGAACCCT
ACACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTTACTTCCA

>contigous sequence

TGGAAGTAAAAAGTCGTAACAAGGTTTCYGCTAGGTGAACCTGCGGAAGGATCATTACCGAGTGTA
GGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTAACCTTAGTTGCTTCGGCGGGCCCGCC
TTTAAGGCCGCCGGGGGGCATCAGCCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCT
GATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTC
CGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATCATCG
AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGC
CCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTTCGGGGGGGACGGGCCCCAAAGGCAG
CGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGC
GCTTGCCGAACGCAAAACAACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATRCCCKCTGA
ACTTAAGCATATCAATAAAGCGGAGGAA

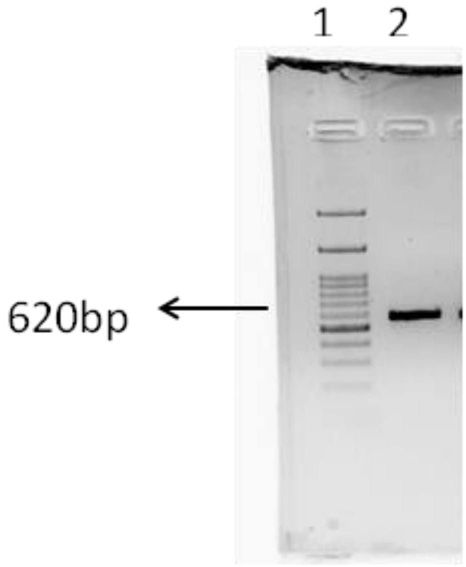

Fig 2: PCR Amplicon of ITS 1-4

Lane 1-Molecular size ladder, lane 2 – Sample *Aspergillus nomiae*

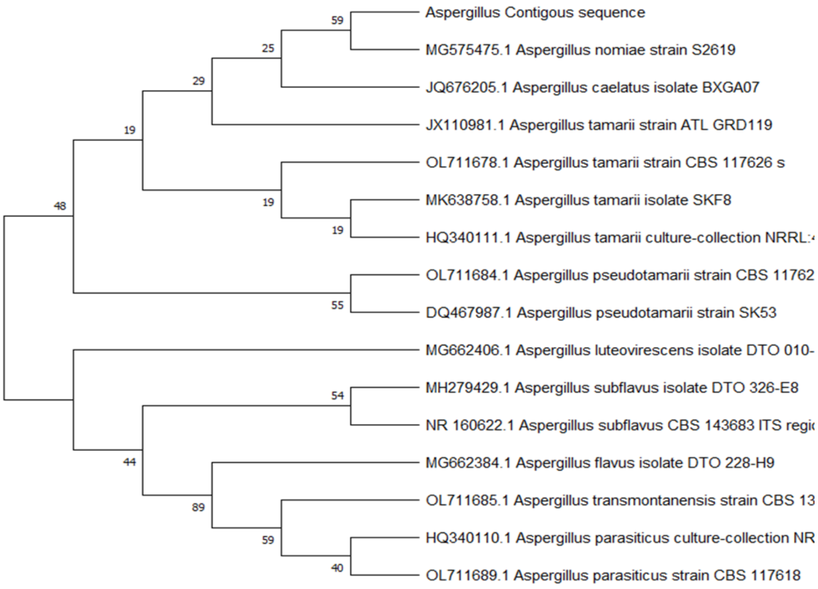

Fig 3: Phylogenetic tree of fungal isolate of *Aspergillus nomiae*

Discussion

In this study, we embarked on a comprehensive genetic analysis of *Aspergillus nomiae* strains isolated from *Sorghum bicolor*, an agriculturally crucial staple crop. Our approach centered on employing Polymerase Chain Reaction (PCR) to amplify and sequence specific genomic regions, providing valuable insights into the genetic diversity and potential pathogenicity of these fungal strains. Our findings unveiled a diverse genetic landscape among the isolated *A. nomiae* strains, indicating the presence of distinct genotypes with potential variations in virulence factors. This diversity is of significant interest, as it could potentially influence the strains' interactions with their environment and hosts. Comparative analysis with existing genomic data sets illuminated unique genomic signatures within the *A. nomiae* strains. These distinctive genetic markers hint at potential adaptations and evolutionary pathways specific to *A. nomiae* populations. Such insights are invaluable in understanding the evolutionary dynamics of this fungal species. Furthermore, our phylogenetic analysis provided a broader perspective on the relationships between the isolated *A. nomiae* strains and other closely related *Aspergillus* species. This insight into the taxonomic and evolutionary history of *A. nomiae* offers a foundational understanding of its place within the fungal kingdom. However, it's important to note that this study was not without its challenges. The presence of genomic complexities, including repetitive sequences and structural variations, presented hurdles during the DNA sequencing process. Additionally, issues related to DNA extraction, purification, and high GC content were addressed through meticulous laboratory techniques.

In examining the phylogenetic relationships of *Aspergillus nomiae* with closely related *Aspergillus* species, it became evident that this was a critical aspect of our study. The genetic relatedness provides invaluable insights into the evolutionary history and taxonomic placement of *A. nomiae*. Through the comparison of genetic markers, we could discern shared ancestry and potential divergence points. One notable observation was the clustering patterns within the phylogenetic tree. It revealed a closer affinity of *A. nomiae* with specific *Aspergillus* species, indicating a probable common ancestor. However, there were also distinct branches, suggesting evolutionary divergence and unique genetic adaptations. Furthermore, this analysis shed light on potential areas of genetic exchange or horizontal gene transfer among these closely related species. It highlighted the dynamic nature of fungal genomes and their ability to acquire genetic material from their ecological niche. Overall, delving into the phylogenetic relationships not only provided clarity regarding *A. nomiae*'s evolutionary history but also offered a broader perspective on the diversity and dynamics within the Aspergillus genus. This understanding holds implications for various fields, from ecology to biotechnology, emphasizing the significance of this research endeavor.

During the DNA sequencing of *Aspergillus nomiae*, several challenges were encountered. One significant hurdle was the presence of genomic complexities within the organism. *Aspergillus nomiae* may possess regions of repetitive sequences or structural variations, making it difficult to accurately sequence these regions. Additionally, issues related to DNA extraction and purification arose, as contaminants or inhibitors could interfere with the sequencing process. Another challenge stemmed from the potential for contamination during sample handling and preparation. Ensuring pure, uncontaminated samples was crucial to obtaining reliable sequencing data. Furthermore, *Aspergillus nomiae* may have a high GC content, which can pose difficulties for certain sequencing platforms. Bioinformatic analysis also presented challenges, particularly in accurately assembling and annotating the genome. Identifying and characterizing specific genes or functional elements within the genome required sophisticated computational tools. Lastly, obtaining sufficient quantities of high-quality DNA from *A. nomiae* strains was a practical challenge. Overcoming these obstacles demanded a combination of advanced laboratory techniques, meticulous sample handling, and sophisticated computational analyses.

The DNA Sequencing of *Aspergillus nomiae* isolated from *Sorghum bicolor* through Polymerase Chain Reaction (PCR) offers a promising foundation for future research avenues. Firstly, expanding the genomic analysis to encompass a broader spectrum of *Aspergillus nomiae* strains from diverse geographical regions could provide deeper insights into its genetic diversity and adaptations. Investigating the functional genomics, including the exploration of key genes and pathways associated with pathogenicity and secondary metabolite production, may unravel potential targets for disease management strategies. Additionally, employing advanced bioinformatics approaches and comparative genomics could uncover unique genomic features and evolutionary trajectories within the *Aspergillus* genus. Exploring the ecological interactions of *Aspergillus nomiae* within the plant

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microbiome, and its role in nutrient cycling, could provide a comprehensive understanding of its ecological significance. Furthermore, the application of cutting-edge sequencing technologies, such as long-read sequencing and metagenomics, may enhance the resolution and depth of genomic analyses. Overall, future research endeavors should focus on integrating multi-disciplinary approaches to advance our comprehension of *Aspergillus nomiae* and its impact on agricultural ecosystems. This knowledge holds great potential for informing effective disease management strategies and contributing to the sustainable cultivation of *Sorghum bicolor*.

Conclusion

In conclusion, our research contributes significantly to the understanding of *Aspergillus nomiae*, shedding light on its genetic makeup, potential pathogenicity, and evolutionary relationships with closely related species. This knowledge is pivotal in agricultural contexts, especially in the management of potential crop diseases caused by *A. nomiae*. Moreover, the utilization of PCR in this study showcases its efficacy as a powerful molecular tool in unraveling the genetic intricacies of fungal pathogens. Overall, this research underscores the importance of genetic studies in elucidating the complexities of agricultural ecosystems.

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