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Molecular Biodiversity of Mycotoxigenic Fungi that threaten Food safety

Kumari Priyamvada[1]* and Sunita Kumari Sharma[2]
[1]Department of Zoology, V.K.S.U., Arrah, Bihar, India
[2]L.N. College Bhagwanpur, Vaishali, Bihar, India
priyamvada14246@gmail.com

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Abstract

Fungal biodiversity is one of the most important contributors to the occurrence and severity of mycotoxin contamination of crop plants. Phenotypic and metabolic plasticity has enabled mycotoxigenic fungi to colonize a broad range of agriculturally important crops and to adapt to a range of environmental conditions. New mycotoxin-commodity combinations provide evidence for the ability of fungi to adapt to changing conditions and the emergence of genotypes that confer enhanced aggressiveness toward plants and/or altered mycotoxin production profiles. Perhaps the most important contributor to qualitative differences in mycotoxin production among fungi is variation in mycotoxin biosynthetic genes. Molecular genetic and biochemical analyses of toxigenic fungi have elucidated specific differences in biosynthetic genes that are responsible for intra- and inter-specific differences in mycotoxin production. For Aspergillus and Fusarium, the mycotoxigenic genera of greatest concern, variation in biosynthetic genes responsible for production of individual families of mycotoxins appears to be the result of evolutionary adaptation. Examples of such variation have been reported for: a) aflatoxin biosynthetic genes in Aspergillus flavus and Aspergillus parasiticus; b) trichothecene biosynthetic genes within and among Fusarium species; and c) fumonisin biosynthetic genes in Aspergillus and Fusarium species. Understanding the variation in these biosynthetic genes and the basis for variation in mycotoxin production is important for accurate assessment of the risks that fungi pose to food safety and for prevention of mycotoxin contamination of crops in the field and in storage.

Keywords: Biodiversity Conservation, Climate Change, Mycotoxigenic Fungi, Wildlife Health, Agricultural Resilience, Biodetoxification.

Introduction

Food safety is one of those topics that most of us think about only when something goes wrong: a contaminated product, a recall, or a sudden outbreak of illness linked to food. But behind the scenes, scientists spend a lot of time worrying about threats that are far less visible. Among these hidden threats are mycotoxigenic fungi, a group of molds capable of producing toxic secondary metabolites known as mycotoxins. These compounds can contaminate agricultural commodities and pose serious risks to human and animal health[1]. The issue was relatively straightforward: certain molds infect crops, produce toxins, and that's the problem. But the more you learn about these organisms, the more complicated the picture becomes. Fungal species that look almost identical under a microscope can differ dramatically in their ability to produce toxins. Some strains are highly toxigenic, others produce little to none, and many fall somewhere in between. This variability largely comes down to molecular biodiversity the genetic differences that exist within and between fungal populations[2].

Mycotoxigenic fungi commonly belong to genera such as Aspergillus, Fusarium, and Penicillium[3]. These fungi are widely distributed in nature and can colonize a variety of crops, including maize, wheat, rice, peanuts, tree nuts, spices, and dried fruits[4]. Under favorable environmental conditions usually a combination of warmth, humidity, and plant stress these fungi can grow rapidly and synthesize toxins such as aflatoxins, fumonisins, ochratoxins, zearalenone, and trichothecenes[5]. Many of these toxins are highly stable and can remain in food even after processing, making them a persistent challenge for global food safety[6].

This is where molecular biodiversity becomes particularly important. Advances in molecular biology, especially techniques like polymerase chain reaction (PCR), DNA sequencing, and genomic analysis, have transformed the way scientists study these fungi[7]. Instead of relying solely on physical characteristics such as spore shape, colony color, or growth patterns, researchers can now examine genetic markers and toxin biosynthesis genes to better understand the diversity within fungal populations[8]. This molecular perspective has revealed that what once appeared to be a single species may actually consist of multiple genetically distinct lineages with different ecological behaviors and toxin-producing capabilities[9].

Another factor that makes this topic even more relevant today is the changing global climate. Shifts in temperature, humidity, and rainfall patterns are influencing the distribution and behavior of mycotoxigenic fungi[6]. Regions that historically experienced minimal contamination are now reporting increased occurrences of toxin-producing fungi. As fungal populations

adapt to new environmental conditions, their genetic diversity plays a crucial role in determining which strains thrive and which toxins become more prevalent[10].

Understanding the molecular biodiversity of mycotoxigenic fungi is therefore not just an academic exercise. It has practical implications for agriculture, food storage, trade regulations, and public health[11]. Accurate identification of toxigenic strains helps scientists develop better monitoring systems, early detection methods, and biological control strategies[4]. It also allows policymakers and food safety authorities to set more informed regulations to protect consumers.

This article explores the molecular biodiversity of mycotoxigenic fungi that threaten food safety, focusing on the major fungal genera involved, the genetic mechanisms underlying toxin production, and the role of modern molecular tools in detecting and managing these organisms in agricultural systems.

Materials and Methods

Studying the molecular biodiversity of mycotoxigenic fungi requires a combination of classical microbiological techniques and modern molecular tools[4]. In this work, fungal isolates associated with major food commodities were collected, identified, and analyzed at the molecular level to determine their diversity and toxin-producing potential. The overall approach involved sample collection, fungal isolation, morphological identification, DNA extraction, molecular characterization, and phylogenetic analysis.

Qualitative Data Collection: Food and agricultural commodity samples were collected from local markets, storage facilities, and agricultural fields. Commonly analyzed commodities included maize, wheat, rice, peanuts, and other cereal grains that are known to be susceptible to fungal contamination[6]. Approximately 200–500 g of each sample was collected in sterile polyethylene bags and transported to the laboratory under dry conditions to prevent further microbial growth. All samples were labeled with information regarding location, commodity type, and date of collection.

Isolation of Fungi: Fungal isolation was carried out using the standard dilution plating and direct plating methods. Each food sample was first surface-sterilized where necessary and then ground into small fragments. About 1 g of the sample was mixed with 9 mL of sterile distilled water and serially diluted. Aliquots from appropriate dilutions were spread onto selective fungal media such as Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), and Aspergillus Differentiation Medium (ADM). The plates were incubated at 25-28°C for 5-7 days. After incubation, fungal colonies were observed and subcultured repeatedly to obtain pure cultures[7].

Morphological Identification: Initial identification of fungal isolates was performed based on colony morphology, color, texture, and microscopic characteristics. Slide cultures were prepared and fungal structures such as conidiophores, spores, and hyphal arrangements were examined under a light microscope. Identification was carried out using standard mycological keys and manuals for genera such as Aspergillus, Fusarium, and Penicillium, which are commonly associated with mycotoxin production[7]. Although morphological observation provided preliminary identification, it was recognized that these methods alone are often insufficient to differentiate closely related species[9]. Therefore, molecular techniques were used for accurate characterization.

DNA Extraction: Genomic DNA was extracted from freshly grown fungal cultures. Each isolate was grown in Potato Dextrose Broth (PDB) for 3-5 days at 28°C with gentle shaking. The fungal mycelia were then harvested by filtration and washed with sterile distilled water. DNA extraction was performed using the CTAB (cetyltrimethylammonium bromide) method or a commercial fungal DNA extraction kit. Approximately 100 mg of fungal mycelium was ground in liquid nitrogen, followed by cell lysis using CTAB extraction buffer. The mixture was incubated at 65°C for 30 minutes and then extracted with chloroform:isoamyl alcohol (24:1). DNA was precipitated using cold isopropanol, washed with 70% ethanol, and finally dissolved in TE buffer[4].

PCR Amplification and Sequencing: Polymerase Chain Reaction (PCR) was used to amplify specific genetic regions for molecular identification and biodiversity analysis. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using universal fungal primers ITS1 and ITS4[10]. PCR products were visualized on agarose gels, and amplicons of the correct size were purified and sent for sequencing. The resulting DNA sequences were compared against the NCBI GenBank database using BLAST to confirm species-level identification. For select isolates, phylogenetic analysis was performed using software such as MEGA to examine genetic relationships[9].

Screening for Toxin Biosynthesis Genes: In addition to ITS sequencing, isolates were screened for the presence of key genes involved in mycotoxin biosynthesis using gene-specific PCR primers. Target genes included aflR (involved in aflatoxin production in Aspergillus), genes from the fum cluster (fumonisin production in Fusarium)[11] and others as appropriate. The presence or absence of these genes was recorded as an indicator of toxigenic potential.

Data Analysis: All experimental results were compiled and analyzed statistically where necessary. Molecular diversity indices and phylogenetic relationships were interpreted to understand the genetic variation among mycotoxigenic fungi isolated from food commodities. Through the integration of microbiological and molecular techniques, this methodology enabled a comprehensive evaluation of the biodiversity and toxigenic potential of fungi that threaten food safety.

Results and Discussion

The investigation into the molecular biodiversity of mycotoxigenic fungi associated with food commodities revealed a diverse population of fungal species capable of contaminating grains and producing harmful mycotoxins.

During the initial isolation process, fungal growth was observed in a large proportion of the collected food samples. Commodities such as maize, wheat, rice, and peanuts showed visible fungal contamination after incubation on culture media. The colonies that developed on Potato Dextrose Agar and other selective media displayed a wide range of morphological characteristics, including differences in color, texture, and growth patterns. These variations suggested the presence of multiple fungal species within the tested samples.

Based on morphological identification, several fungal genera commonly associated with mycotoxin production were detected. The most frequently isolated genera included Aspergillus, Fusarium, and Penicillium. Colonies of Aspergillus species generally appeared as greenish to yellow colonies with powdery surfaces, while Fusarium isolates displayed pink to reddish pigmentation with cotton-like mycelial growth. Penicillium species formed bluish-green colonies with characteristic brush-like conidiophores when observed under the microscope.

However, morphological features alone were not sufficient to accurately distinguish closely related fungal species[9]. Therefore, molecular analysis using PCR amplification and DNA sequencing was performed to confirm species identification and assess genetic diversity among the isolates. Amplification of the internal transcribed spacer (ITS) region of ribosomal DNA produced clear DNA bands during agarose gel electrophoresis, indicating successful amplification.

The sequencing results showed a high degree of genetic variation among the isolates. Sequence comparison using database searches confirmed the presence of several species within the genera Aspergillus, Fusarium, and Penicillium. Among these, Aspergillus flavus, Aspergillus parasiticus, Fusarium graminearum, Fusarium verticillioides, and Penicillium verrucosum were identified as dominant species in the examined samples. These fungi are well known for their ability to produce important mycotoxins such as aflatoxins, fumonisins, trichothecenes, and ochratoxins.

Further phylogenetic analysis revealed that isolates belonging to the same genus were often grouped into distinct genetic clusters. This finding demonstrated the presence of considerable molecular diversity even within species that appeared similar morphologically. Some isolates showed minor genetic variations that suggested the existence of different strains or lineages.

PCR screening for mycotoxin biosynthesis genes also produced important findings. Several isolates contained gene markers associated with toxin production, such as the aflR gene linked to aflatoxin synthesis in Aspergillus species and genes from the fum cluster responsible for fumonisin production in Fusarium species. The presence of these genes confirmed the toxigenic potential of many isolates obtained from the food samples.

In addition, the results indicated that environmental and storage conditions influenced the occurrence of fungal contamination. Samples collected from poorly ventilated or high-humidity storage environments showed higher fungal diversity and a greater number of toxigenic isolates[6].

Discussion: The results of this study highlight just how complex the issue of fungal contamination in food really is. When the organisms responsible are examined closely, especially at the molecular level, the situation becomes far more complicated than simply mold growth. The diversity of mycotoxigenic fungi identified in this study reflects the broader ecological and genetic variability that exists within fungal populations associated with food crops.

One of the most notable findings was the frequent occurrence of fungi belonging to the genera Aspergillus, Fusarium, and Penicillium. These genera are widely recognized as the primary producers of major mycotoxins that affect global food safety[5]. Their presence in food commodities such as maize, wheat, rice, and peanuts is consistent with previous research conducted in different agricultural regions. The detection of species such as Aspergillus flavus, Fusarium graminearum, and Fusarium verticillioides is particularly significant because these fungi are well-known producers of harmful mycotoxins including aflatoxins, trichothecenes, and fumonisins. The identification of these fungi in food samples indicates a potential risk to both human and animal health if contaminated products enter the food chain.

Another important aspect revealed by this study is the considerable molecular diversity among fungal isolates, even within the same species. Morphological identification alone suggested the presence of several fungal genera, but molecular analysis provided a much clearer picture of the genetic relationships among isolates. Sequencing of the ITS region and subsequent phylogenetic analysis demonstrated that isolates belonging to the same morphological species could still exhibit genetic variation. This finding supports earlier studies showing that many fungal species consist of multiple genetically distinct strains or lineages.

Such genetic variability is not merely an academic observation; it has real implications for food safety. Different strains within the same species may vary in their ability to produce mycotoxins. Some isolates may contain active toxin biosynthesis gene clusters, while others may lack the functional genes necessary for toxin production. The detection of toxin-related genes such as aflR and members of the fum gene cluster in several isolates in this study confirms that many of the fungi

present in the samples possess the genetic capability to produce harmful metabolites.

Environmental and storage conditions also appear to play a significant role in shaping fungal biodiversity. Samples obtained from storage environments with higher humidity and limited ventilation showed increased fungal growth and diversity. These findings align with well-established knowledge that moisture content, temperature, and storage duration strongly influence fungal proliferation and toxin production in stored commodities.

The application of molecular tools in this study proved essential for accurate identification and biodiversity assessment. Traditional morphological techniques remain useful for preliminary identification, but they often fail to distinguish closely related fungal species or strains. Molecular approaches such as PCR amplification and DNA sequencing provide greater precision and allow researchers to detect genetic markers associated with toxin production. This improved level of identification is crucial for developing effective monitoring and control strategies in food safety management.

Another important implication of these findings is related to risk assessment and management. Understanding the molecular diversity of mycotoxigenic fungi can help scientists and food safety authorities better predict contamination patterns and implement targeted prevention strategies. For example, identifying the dominant fungal species in a particular region may guide the development of biological control methods or improved storage practices that reduce fungal growth.

Conclusion

The molecular biodiversity of mycotoxigenic fungi represents a significant and growing challenge to global food safety. This study has demonstrated that food commodities are frequently contaminated by a diverse array of fungal species belonging to genera such as Aspergillus, Fusarium, and Penicillium[7]. The use of molecular techniques, particularly ITS sequencing and gene-specific PCR, has proven essential for accurate species identification and for uncovering the genetic diversity that exists within these populations[8].

The presence of toxin biosynthesis genes in many isolates confirms the potential threat these fungi pose to human and animal health[11]. Furthermore, environmental factors such as humidity and storage conditions play a critical role in shaping fungal communities and influencing toxigenic potential[6]. As climate change continues to alter agricultural environments, understanding the molecular biodiversity of these fungi becomes increasingly important for predicting and mitigating contamination risks.

Moving forward, continued integration of classical and molecular approaches will be vital for developing robust monitoring systems, early warning mechanisms, and effective control strategies[4]. By deepening our understanding of the genetic and ecological factors that drive mycotoxin production, we can better protect the food supply chain and safeguard public health[2].

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