Morphometric and Genomic Characterization of Toxigenic Aspergillus Isolates Associated with Poultry Feeds and Feed Ingredients from Five Agro-Ecological Zones of Nigeria

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Authors’ contributions

This work was carried out in collaboration between both authors. Author AOO designed the study, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author OIO managed the analyses of the study. Both authors read and approved the final manuscript.

ABSTRACT

Introduction: Toxigenic aspergilli are storage contaminants of poultry feeds and feed ingredients which produce secondary metabolites known as mycotoxins in these products. This research is aimed at detecting and characterizing mycotoxin producing aspergillus species present in feeds given to poultry birds and in the ingredients used in the production of these feeds. Method: A total of 102 samples of feed (n=30) and feed ingredients (n=72) were collected across 5 agro-ecological zones of Nigeria and analyzed for toxigenic Aspergillus using morphometric and molecular genomic characterization techniques. Different mycological media was used for isolation, by deploying pour plate method. The Aspergilli isolates morphometric identity were characterized using macroscopic and microscopic observed features. The isolated Aspergillus species were further characterized molecularly using genomic characterization protocols. Results: The results of the morphometric characterization indicated that nine (9) Aspergillus species were detected in the feed and feed ingredients, with Aspergillus flavus having the highest rate of occurrence among the isolates. Furthermore, the genomic typing using internal transcribe

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spacer (ITS) as a barcode, as well as comparison of the fungal isolates with other Aspergillus species in the genebank showed 99-100% similarity. Also, toxigenic gene typing indicated that some of the A. flavus were toxigenic with the detection of the following genes Nor-1, ver-1, OmtB and AflR.

**Conclusion:** The findings of this study showed that toxigenic Aspergilli isolates are present in both poultry feed and feed ingredients from 5 agro-ecological zones in Nigeria and poses great public health issues.

**Keywords:** DNA extraction; genomic characterization techniques; ITS region; locally compounded feeds; mycotoxins; polymerase chain reaction; toxigenic fungal isolates.

### 1. INTRODUCTION

In Nigeria, the poultry sector is the most commercialized agricultural subsector. It solves the problem of malnutrition in the shortest possible time by providing animal protein. Poultry feeds originate from animal and plant sources and are commonly contaminated with microorganisms during harvesting, processing, storage, transport and packaging. International legislation on food and feeds was established by Codex Alimentarius Commission (CAC). This commission defines contamination as any substance not intentionally added to food which is present in such food as a result of production. This includes operations carried out in crop production, animal husbandry, Veterinary Medicine, manufacture, processing, preparation, treatment, packaging, loading, transport or holding of such food or as a result of environmental contamination. This definition by codex invariably includes naturally occurring toxicants which include toxic metabolites of certain micro fungi [1]. Aspergillus species are found throughout the world, being present in both the soil and the air and have been discovered to be the most common moulds involved in mycotoxin production [2]. These aspergilli reduce feed quality and the mycotoxins they produce can hinder productivity when ingested by poultry.

When carried over into meat and eggs could have carcinogenic, haemorrhagic, hepatotoxic and nephrotoxic effects in man [3].

There is therefore the need to determine the Aspergillus mycobiota of poultry feeds and their raw materials in Nigeria. A proper identification and characterization of aspergillus species in poultry feeds and their ingredients will provide insight into the array of mycotoxins produced by these species Aspergillus species such as Aspergillus terreus, Aspergillus flavus, Aspergillus niger, and Aspergillus fumigatus. Both morphological and molecular methods must be employed in order to properly identify aspergillus species therefore the internally transcribed spacer region (ITS) and phylogenetic analysis were used for identification.

### 2. MATERIALS AND METHODS

#### 2.1 Survey and Sample Collection location Description

A total of 102 samples of locally compounded poultry feed and the raw materials used for their production were collected for analysis. The samples were collected from selected farms across the five agro-ecological zones within a period of 30 days. One kilogram (1 Kg) of each sample was collected manually from three points (top, middle and bottom) of the bulk feed / ingredient bag using a probing pointer. The three 1 Kg samples collected from each bulk feed / ingredient bag were mixed thoroughly and a subsample measuring 1 Kg was collected in zip lock bags to form each sample lot. Samples were transported to the laboratory and stored at 4°C to prevent additional liberation of metabolite by fungi before further analysis.

#### 2.2 Isolation and Phenotypic Identification of Fungi

Using the dilution plating method [4], a 10 g representative sample per bulk was comminuted, diluted in 90 ml sterile water and 1 ml of this was seeded onto each plate of Rose Bengal Chloramphenicol Agar (RBCA), Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Corn Meal Agar (CMA). Plates were incubated at 25°C. After the first 48 hours, plates were examined daily for seven (7) days. All fungi considered to represent different species were then sub cultured onto Potato Dextrose Agar (PDA) plates for phenotypic identification Isolates
were identified through macroscopic observations (colour and nature of the fungal growth both on the surface of the growth medium and on the reverse side) and microscopic observations (nature of hyphae, presence or absence of spores and other microscopic structures), with the aid of a light microscope using x10 and x40 objective lens and published guidelines [5,6].

2.3 Molecular Characterization of Fungal Isolates

2.3.1 DNA extraction

Monospore isolates grown on petri dishes were used for DNA extraction. For each sample 200 mg of mycelium from a 7-day culture was grinded into powder in liquid nitrogen. DNA was extracted following the protocol of [7] and stored at -20°C.

2.3.2 Polymerase chain reaction assay

The DNA amplification (PCR reactions) was carried out using universal primers ITS1 (5'-TCCGTAGGTTGAACTTGCGGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3').

(Table 1) [8]. The amplified fragments include: ITS1, 5.8S and the ITS2 of rDNA. The PCR reaction was performed in a 30 μl reaction mixture containing 10 mM Tris–HCl (pH 9.0), 50 mM MKCl, 0.1% Triton X 100, 2 mM MgCl2, 0.2mM dNTPs, 0.4 μM primers, using 0.5-unit EF-Taq polymerase (SolGent, Korea) and 50 ng of fungal genomic DNA as template. The thermal cycling programme used include activation of Taq polymerase, followed by cycles of denaturation, primer annealing and extension (Table 2). The products of PCR were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA).

The amplicons were separated by electrophoresis on a 1.5% (w/v) agarose gel, stained with ethidium bromide and visualized using ultra-violet (UV) light.

2.3.3 DNA sequencing

Direct DNA sequencing was performed using primers ITS3 (5'-GCATCGATGAAGAACCCGCGGC-3') and ITS5 (5'-GGAAGTAAAAGTCCGTAACAAGG-3') [8] using PRISM Big Dye Terminator v3.1 Cycle sequencer.

![Fig. 1 Distribution of sampling location (five agro-ecological zones) in Nigeria](image-url)
Table 1. Oligonucleotide primers used for molecular identification and sequencing of *Aspergillus* isolates

| Primer | Sequence(5’3’) | Amplified Product | Reference |
|--------|----------------|-------------------|-----------|
| ITS1-F | 5’TCCGTAGGTGAACCTGCGG-3’ | 570-600bp | [8] |
| ITS4-R | 5’-TCCTCCGCTTATGATAGC-3’ | | |

Table 2. Thermal profile and cycling conditions used for PCR

| Target | Primary Denaturation | Secondary Denaturation | Annealing | Extension | No. Of Cycles | Final Extension |
|--------|----------------------|------------------------|-----------|-----------|--------------|----------------|
| ITS region | 95 °C for 5 minutes | 94 °C for 30 seconds | 56°C for 45 seconds | 72 °C for 1 minute | 35 | 72 °C for 7 minutes |

2.3.4 DNA sequence analysis

All of the representative fungal sequences were aligned and edited using the MEGA software (Muscle program) version 7. The nucleotide sequences were compared and analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

2.3.5 Gene detection

Detection of target gene responsible for the production of Aflatoxins and Ochratoxins using multiplex PCR, in *Aspergillus flavus* and *Aspergillus niger* respectively was carried out with forward and reverse primers of Aflr [9] OmtB, nor-1, ver-1, [10], OCRA-1, [11], AOPKS-1 [12] (Table 3) and the optimized cycling conditions employed are shown in (Table 4).

A 2 μL aliquot of each amplification product was then analysed in 1.5% (w/v) agarose gels running at 300 V and 200 A for 20 minutes. Gels were stained with ethidium bromide (0.5 mg/mL, Sigma Aldrich) and visualised by UV trans illumination G-Box of Syngene. They were photographed by means of the camera and software equipment, Gene Snap and Gene Tools, by Syngene. A DNA molecular size marker of 100 bp (Thermo Scientific Gene ruler DNA Ladder mix) was used to determine the size of the PCR products.

3. RESULTS

The morphometric characterization indicated the presence of nine aspergillus species. Table 5 and plate 1 shows macroscopic and microscopic the characteristics of these Aspergillus species.

The PCR amplification of the 500 bp ITS region from genomic DNA of *Aspergillus* species isolated from compounded poultry feeds and feed ingredients is presented in plate 2. Lane 1 represented the molecular ladder. Ten isolates of *Aspergillus* species which appeared on lane 2-11 were positive while Lane 12 and 13 were positive and negative controls respectively.

The PCR amplification of the genes Ver-1, Nor-1, OmtB and Aflr from genomic DNA of *Aspergillus flavus* strains, Ocra-1 gene and Aopks-1 from the genomic DNA of *Aspergillus niger* isolated from compounded poultry feeds and feed ingredients is presented in plate 3 and Plate 4. The molecular weight marker was found on Lane M, Ver-1 gene appeared on lane 1, OmtB gene appeared on lane 2, Nor-1 appeared on lane 3 and Aflr appeared on lane 4. These genes amplified at 537 bp, 611bp, 400 bp, and 698 bp respectively in the 3 positive strains of *Aspergillus flavus*. Aopks-1 gene and Ocra-1 gene from the genomic DNA of *Aspergillus niger* on lane 5 and 6 respectively were negative as they did not amplify.

4. DISCUSSION

The contamination of grains and feeds by Aspergillus species is a global problem with an overwhelming economic impact worldwide. The growth of Aspergillus is unfavourable in to the development of the food and feed industries due to the production of mycotoxins by these fungal species [3].

The isolation of these different species of Aspergillus from the feed samples is a confirmation that the genus is a wide spread storage contaminant of grains and their products which have been implicated in the production of mycotoxins making such grains and their products have serious health implications when consumed by man or animals [13].
Table 3. Oligonucleotide primers sequences to detect Aflatoxigenic and ochratoxigenic *Aspergillus* species

| Primer  | Primer Sequences                                      | Molecular Weight (bp) | Gene                  |
|---------|-------------------------------------------------------|-----------------------|-----------------------|
| OmtB-1  | Forward (5’TCCAGAACACGATGTGG-3’) Reverse (5’-CGTTGGCTAGAGTTTGGAGG- 3’) | 611bp                 | Aflatoxin OmtB       |
| OmtB-2  | Forward (5’ CTTCCATTAGGGTGCCACAGC 3’) Reverse (5’ GTTGCTTTTTCAGCCTCGCC 3’) | 400b                  | rRNA (ITS region)    |
| OCRA-1  | Forward (5’ CAGACCATCGACACTGCATGC 3’) Reverse (5’ CTGGGCGTTCCATACGAG 3’) | 549bp                 | Polyketide synthase  |
| AOPKS-1 | Forward (5’ CTGGCGTTCCATACGAG 3’) Reverse (5’ CAGACCATCGACACTGCATGC 3’) | 698bp                 | Aflatoxin AflR       |
| AOPKS-2 | Forward (5’ AAGTGAATGGCCGAGACG 3’) Reverse (5’ CTACCTGCTCATCGGTA-3’) | 537bp                 | Aflatoxin Ver-1      |

Table 4. Thermal profile and cycling conditions used during PCR

| Primary Denaturation | Secondary Denaturation | Annealing | Extension | No. Of Cycles | Final Extension |
|----------------------|------------------------|-----------|-----------|---------------|----------------|
| 95 °C for 5 minutes  | 95 °C for 30 seconds   | 55 °C for 30 seconds | 72 °C for 1 minutes | 35            | 72 °C for 7 minutes |

Table 5. Morphometric characteristics of Toxigenic *Aspergillus* species isolated from poultry feeds and feed ingredients

| S/N | Macroscopic Observations                                                                 | Microscopic Observations                                                                                       | Name of Isolate        |
|-----|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|------------------------|
| 1.  | Colonies on potato dextrose agar plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse brown. | Conidiophore stipes are smooth-walled, hyaline and brown in colour. Phialides are borne on metulae. Conidia globose and rough walled. | *Aspergillus niger*    |
| 2.  | Colonies on Potato dextrose agar attained a diameter of 3-5 cm within 7 days. It consist of a dense felt of yellow-green conidiophores | Conidiophores are hyaline and coarse, vesicles are globose. Phialides are borne directly on the vesicle. Conidia are globose to sub globose pale green and echinulate. | *Aspergillus flavus*   |
| 3.  | Colonies on potato dextrose agar attained a diameter of 4-5cm within 7 days consisting of a dense felt of yellow - brown conidiophores which rapidly turned dark green-brown. | Conidiophores are hyaline and smooth walled. Vesicles are sub globose and phialides are borne directly on vesicle. | *Aspergillus tamarii*  |
| 4.  | Colonies on potato dextrose agar plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse brown. | Conidiophores are short, smooth walled and brownish. Phialades are borne on metulae. Conidia are globose and rough-walled. Mass of hülle cells are present. | *Aspergillus nidulans* |
| 5.  | Colonies on Potato dextrose agar attained a diameter of 3-5 cm within 7 days consisting | Conidiophores are short, smooth walled and green. Vesicles are clavate. Phialides are directly | *Aspergillus fumigatus* |
of a felt of dark green conidiophores mixed with aerial hyphae.

6. Colonies on potato Dextrose Agar attained a diameter of 3.5-5.0cm at 7days consisting of dense felt of yellow brown conidiophore, Conidiophores are hyaline and smooth walled. Vesicles are subglobose and phialades are borne on metulae. Conidia are globose, hyaline and smooth.  

Aspergillus terrus

7. Colonies on Potato dextrose agar attained a diameter of 4-5cm at 7days, sporulating densely with a felt of conidiophores intermixed with aerial mycelia. Colour is pale greenish yellow becoming light to dull brown. Conidiophores are hyaline and rough walled, vesicles are subglobose. Phialades are borne directly on the vesicle or metulae. Conidia is globose to subglobose with rough wall. 

Aspergillus ochraceus

8. Colonies on Potato Dextrose agar at 7 days sporulating densely with a dense felt of yellow brown conidiophores Conidiophore stalk is hyaline and rough walled. Vesicles are globose to sub globose. Phialades are borne on metulae or directly on the vesicle. Conidia are globose to subglobose with rough wall. 

Aspergillus parasiticus

9. Colonies on potato dextrose agar attained a diameter of 4-5 cm within 7days consisting of a dense felt of conidiophores. It is greenish yellow, becoming dull brown with age. Conidiophores are hyaline and smooth walled. Vesicles are subglobose and phialades are borne on metulae.

Aspergillus oryzae

| S/N | Name of isolates | Macroscopic obverse view | Macroscopic reverse view | Microscopic features |
|-----|------------------|--------------------------|--------------------------|----------------------|
| 1.  | Aspergillus niger | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| 2.  | Aspergillus flavus | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| 3.  | Aspergillus tamari | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| No. | Species                  |
|-----|-------------------------|
| 4.  | *Aspergillus nidulans*  |
| 5.  | *Aspergillus fumigatus* |
| 6.  | *Aspergillus terreus*   |
| 7.  | *Aspergillus ochraceus* |
| 8.  | *Aspergillus parasiticus* |
| 9.  | *Aspergillus oryzae*    |

Plate 1. Toxigenic *Aspergillus* species isolated from poultry feeds and feed ingredients.
Plate 2. The PCR amplification of the ITS region from genomic DNA of *Aspergillus* species isolated from compounded poultry feeds and feed ingredients
Lane 1 molecular ladder, Lane 2 to 11 positive isolates of *Aspergillus* species, lane 12, positive control, lane 13 negative control

Plate 3. PCR amplification on 1.5 % agarose gel for Ver-1 gene, omtB gene, Nor-1 gene and AflR gene of *Aspergillus flavus* (2) strains
*M*= molecular maker, Lane 1= Ver-1, Lane 2= OmtB, Lane3= Nor-1, Lane 4= AflR

Plate 4. PCR amplification on 1.5 % agarose gel for Ver-1 gene, omtB gene, Nor-1 gene and AflR gene of 1 strain of *Aspergillus flavus*. Aopks-1 and Ocra-1 genes of *Aspergillus niger* were negative
*M*= molecular maker, Lane 1: Ver-1, Lane 2: OmtB, Lane3: Nor-1, Lane 4: AflR. Lane 5: Aopks-1, Lane 6: Ocra-1 gene
The genus *Aspergillus* was isolated from 100% of the feed samples studied. *Aspergillus flavus* and *Aspergillus niger* were isolated from 100% of the compounded feeds, making them the most predominant species in the samples. *Aspergillus flavus* are the most significant producers of aflatoxins which are known class one carcinogens [14]. This result is in line with the findings of [15] and [16] who reported *A. flavus* and *A. niger* as the most predominant contaminants in poultry feeds in south west Tunisia and Iran respectively. Similarly, the results of this study are also in consonance with the work of [17], [9] and [18] who reported *A. flavus* as the most predominant species followed by *A. niger*. This differs from the work of [19] which describe *Aspergillus niger* as the most frequently isolated species in feed samples and ingredients. Other aspergillus species isolated from feeds in this present study include *A. tamarii, A. nidulans* and *A. fumigatus, A. terreus, A. ochraceous, A. parasiticus* and *A. oryzae*. These species were also isolated from poultry feeds by [20] and [21] in Argentina and [22] from poultry feed ingredients and finished feeds in Iran.

Similar results were also reported by [18] in Brazil, and [23] in Iraq, they reported that the *Aspergillus* genera was a very common contaminant of poultry feeds.

The results of this study showed that the reference strains of *Aspergillus* species obtained from the GenBank (National Center for Biotechnology Information database) showed 99.0% to 100.0% sequence identity with the Nigerian isolates. This showed a high level of similarity among the strains worldwide. This high degree of similarity showed how closely related the strains of these isolates are worldwide. This result is similar to that of [8] who used the ITS region for the identification of fungi and obtained strains with 99% to 100% sequence identity with reference strains. This result further confirms the ITS (ITS1, 5.8S, ITS2) region of the rRNA for these fungi as the best tool for their identification. The region contains the most conserved sequence at the terminal region and also contains the hyper variable sequences distinguishing between species.

Polymerase chain reaction showed the existence of the gene (OmtB) responsible for the production of aflatoxins in the three isolates of *A. flavus*. The location of the gene appeared at the nitrogenous base (611 bp). This result is in line with the findings of [10] and [24] who detected the presence of OmtB gene at 611bp using PCR in their study. The existence of a gene (nor-1) of three isolates of *A. flavus* at the nitrogenous base, 400 bp, is in agreement with the report of [25] in 7 strains of *Aspergillus flavus* associated with production of aflatoxins in Iran. Similarly, a study conducted in India by [26] detected nor-1 gene from 60 out of 89 isolates of *A. flavus*. Bands of the fragments of ver-1 and aflR genes were visualized at 537 and 698 bp respectively in line with [25]. All the three *A. flavus* isolates showed DNA fragments that corresponded to the complete set of target aflatoxinogenic genes. The presence of four targeted genes OmtB, nor-1, ver-1 and AflR confirms the ability of the isolates to produce aflatoxins as stated by [27], [28],[29] and [30]. This shows that the *A. flavus* strains isolated in this work were all aflatoxinogenic strains. The *Aspergillus niger* strain investigated for ochratoxin production in this study did not show any amplification after undergoing PCR with the forward and reverse primers Ocra and Aopks for the amplification of Ocra and Aopks gene responsible for the production of ochratoxins. This is in line with the result obtained by [28] who stated that the *A. niger* isolates that were unable to amplify the Ocra and Aopks gene were non-ochratoxigenic strains. These genes might have been lost through the process of genetic mutation or did not originally possess the genes as it obtains in non-toxigenic strains of *Aspergillus niger* that are generally regarded as safe (GRAS) in the food industry.

5. CONCLUSION

This research work has been able to bring to lime light the presence of mycotoxigenic fungi in compounded poultry feeds and the ingredients used in production of these feeds in some parts of Nigeria and this signifies a high level of contamination of both the feeds and ingredients during harvest and storage. There is a need for further studies to be carried out on the both the ingredient and feed production line so as to determine the areas where critical control should be applied.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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APPENDIX

Result of DNA Sequencing Analysis

*Aspergillus terreus* isolate UNIMAID 02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

**ACCESSION:** MG640041

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 1 GCACCGGGGCC GGGCCGGGTC GCCCCCCGGC GGCAGAAAC GCCGCGGCGC
CGCGGGAAGC
 61 AACCAGGTAC CAAAGTCAGG GGGGGAGGTT GGGCCATAAA GACCCGCACT
CGGTAATCT
121 TTCCGGAAG GGGGAATGCG GAAGGATCAT TACCGAGTGC GGGTCTTTAT
G GCCCAACCT
181 CCCACCCTGT ACTATTG TAC CTTGTTGCTT CGGCGGGCCC GCCAGCGTTG
CTGGCCGCCG
241 GGGGGCGACT CGCCCCCGGG CCCGTGCCCG CCGGAGACCC CAACATGAAC
CTTCTTCTGA
301 AAGCTTGCAG TCTGAGTGTG ATTCTTTGCA ATCAGTTAAA ACTTCCAACA
ATGGATCTCT
361 TGGTTCCGCC ATCGATGAAAG AACGCAGCAG AATGCGGATA AATAGTGAAC
TTCCGGAATT
421 CAGTGAATTC TCGAGTCTTT GAACGCACAT TGCGCCCCCT TTGGGCCCTC
481 GGTCGCCGCG TGATTGCTGC CCTCAAGCCC GGCTTGTGTG TTGGGCCCTC
541 TCCCCGGGGA AGGCAGCGGA AGGCAGCGCT CGGTCCTCCTG
AGCTATGGG
601 GCTTCTTCTT CCGCTCCG
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*Aspergillus* sp. isolate UNIMAID 03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. **ACCESSION:** MG640042

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 1 GAAGCAACCC AGGTACCCAA AGTCCAGGGG TGGGAGGTTG GGCCATAAAA
ACCCGGCCTC
61 CGGTCTTCTT TCCCAAAGAG GGGGAATGCG GAAGGATCAT TACCGAGTGC
GGGTCTTTAT
121 GCCCAACCTC CTATTGACC TTGTTGCTTC GGCGGGCCCG CCAGCGTTGC
181 TGGCCGCCGG GGGGCG ACTC GCCCCCGGGC CCGTGCCCGC CGGAGACCC
241 CTGGTTCCGCC ATCGATGAAAG AACGCAGCAG AATGCGGATA AATAGTGAAC
CCAGTGAAC
241 CAGTGAATTC TCGAGTCTTT GAACGCACAT TGCGCCCCCT TTGGGCCCTC
421 GGTCGCCGCG TGATTGCTGC CCTCAAGCCC GGCTTGTGTG TTGGGCCCTC
481 TCCCCGGGGA AGGCAGCGGA AGGCAGCGCT CGGTCCTCCTG
AGCTATGGG
601 GCTTCTTCTT CCGCTCCG
```

*Aspergillus tamarii* isolate UNIMAID 04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. **ACCESSION:** MG640043

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 1 GAAGCAACCC AGGTACCCAA AGTCCAGGGG TGGGAGGTTG GGCCATAAAA
ACCCGGCCTC
61 CGGTCTTCTT TCCCAAAGAG GGGGAATGCG GAAGGATCAT TACCGAGTGC
GGGTCTTTAT
121 GCCCAACCTC CTATTGACC TTGTTGCTTC GGCGGGCCCG CCAGCGTTGC
181 TGGCCGCCGG GGGGCG ACTC GCCCCCGGGC CCGTGCCCGC CGGAGACCC
241 CTGGTTCCGCC ATCGATGAAAG AACGCAGCAG AATGCGGATA AATAGTGAAC
CCAGTGAAC
241 CAGTGAATTC TCGAGTCTTT GAACGCACAT TGCGCCCCCT TTGGGCCCTC
421 GGTCGCCGCG TGATTGCTGC CCTCAAGCCC GGCTTGTGTG TTGGGCCCTC
481 TCCCCGGGGA AGGCAGCGGA AGGCAGCGCT CGGTCCTCCTG
AGCTATGGG
601 GCTTCTTCTT CCGCTCCG
```
Aspergillus oryzae isolate UNIMAID 05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. ACCESSION MG640044

Aspergillus nidulans isolate UNIMAID 06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. ACCESSION MG640045
Aspergillus flavus isolate UNIMAID 10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. ACCESSION MG640049

Aspergillus flavus isolate UNIMAID 01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. ACCESSION MG719379
Aspergillus niger isolate UNIMAID 02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2 partial sequence. ACCESSION MG719380

1 GGCGGCCGAC CAGAGGCGGG CCCGCCGAAG CAACAGGGTC CCAAAGACAG
   GGATGGGAGG
61 TGGGCCCAAA GGACCCGCAC TCGGTACTGA CTTTCCGAGG GGGGGACTGC
GGAAAGGATCA
121 TTACCGAGTG CCGGTCTCTT GGGCCCAACC TCCCATCCGT GTCTATTGTA
CCCTGTTGCT
181 TCGGCGGGCC CGCGGTTTG CCGGCCCGGG GGGGCCGGCT CTGCCCCCGG
GGCCGGTGCC
241 CGCGCGAGAC CCCAACACGA ACACCTGCTG AAAGCGTGCA GTCTAGTG
   247 ATTGAATGCA
301 ATCAGTTAAA ACTTTCAACA ATGGATCTCT TGGTTCCGCG ATCGATGAA
   307 AACGCAGCGA
361 CATTGCCAGAC CTAGTGTGAA TTGCAGAATT CAGTGAATCA TCGAGTCT
   367 GCACCGCGTC CGATCCTCGA CGCGTATGGG CTTTTGTCAC CC

Aspergillus flavus isolate UNIMAID 03 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. ACCESSION MG719381

1 CTGCCGAAGG ATGTACGGGA ATGTAGGGT TCCTAGCGAG CCCAACCTCC
   CACCCGTGTT
61 TACTGTACCT TAGTTGCCTC GGCAGGGCCG CCGGCGCCTT CTCTCAGCCC
   CGGGCCCGCG CCAGGCCGAG ACACCACGAA CTCTGTCTGA TCTAGTG
   121 TCTCAGCAGG
   181 ATCAGGTAAA ACTTTCAACA ATGGATCTCT TGGTTCCGCG
   241 AACGCAGCGA CTAGTGTGAA TTGCAGAATT CAGTGAATCA TCGAGTCT
   301 TCGAGTCTCT TCGGCCCCCT GGTATTCCGG GGGGCATGCC TGTCCGAGC
   361 TGCGCCCCCT GGTATTCCGG GGGGCATGCC TGTCCGAGCG TCATTGCTGC
   CCATCAAGCAC CGGCTTGTGT GTTGGTCCGG GTCCCCCTCT CGGCCCCGAC GGGGGCG
   421 CGGCCCCGAC GGGCCCCGAC GCCGAGTCCGTCTGCTC GCGTATGGGG CTTTTGTCAC CC

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