Nwanna, L. C.; Ajani, E. K.; Omitoyin, B. O.; Magawata, I. Ayoola, S. O.; Orisasona, O.; Kareem, O. K and Abubakar, M. Y. (Eds.) (2004) ***Proceedings of the Fifth Conference of Association of Nigerian Fisheries Scientists.* "SUSTAINABLE FISHERIES AND AQUACULTURE PRODUCTION FOR ENHANCING LIVELIHOOD IN NIGERIAN ECONOMIC DEVELOPMENT"** 9TH – 11TH JULY, 2024 In the University of Lagos, Lagos, Nigeria

# Isolation and Characterization of *Aspergillus aculeatus* strain CUAB-OLU from *Penaeus monodon* Obtained from Olomore Market, Abeokuta North Local Government, Ogun State

***Adedeji F. A.[1], Ogundare O. S[2] and Oyelami O. I.[3]**

Department of Biological Sciences, Crescent University Abeokuta.

Corresponding Author: adedeji.fatai@cuab.edu.ng, +2348033135761

**Abstract**

Like other seafood, prawns are vulnerable to microbial contamination, which can arise from various sources throughout their lifecycle, including environmental exposure, handling, processing, storage, and distribution. Such contamination can significantly impact the quality, safety, and shelf-life of prawn products, and pose potential health risks to consumers. This research aimed to isolate and characterise the fungi present in prawns obtained from Abeokuta Ogun state, Nigeria. Fresh prawn samples were purchased at Olomore market in Abeokuta, Ogun state. The fungal count was determined. The isolated fungi were identified using cultural and microscopic characterisation. The percentage frequencies were determined, and the dominant fungal isolate was characterised using molecular method. The result showed that the skin of prawn consistently had the highest count at 4 x $10^5$ CFU/g. The counts for the head and gut were lower, with the gut having the least at 1 x $10^5$ CFU/g. The fungi isolates were identified as *Mucor spp* (20%) and *Aspergillus spp*. (80%) which was specifically identified as *Aspergillus aculeatus* strain CUAB-OLU. This novel strain had 583 base pairs, along with seventeen unique restriction sites and shared genetic relationships with *Aspergillus* strains catalogued in the NCBI database. The detection of high fungal counts emphasised the need for improved handling and storage practices to prevent potential health risks associated with mycotoxin production.

**Keywords:**

*prawns, CUAB-OLU, microbial contamination*

## Introduction

Aquaculture has become an increasingly important sector in the global food production system, providing a significant source of protein and contributing to food security (FAO, 2020). Prawns, also known as shrimp, are one of the most extensively cultivated and commercially valuable aquatic organisms worldwide. The species *Penaeus monodon*, commonly referred to as the giant tiger prawn or black tiger shrimp, is a widely farmed prawn species due to its high market demand and nutritional value (Briggs et al., 2004). However, the aquaculture industry faces various challenges, including disease outbreaks caused by microbial pathogens. Fungi are one of the major groups of microorganisms that can infect and cause diseases in prawns, leading to significant economic losses for farmers (Oidtmann, 2012). Fungal infections in prawns can affect their growth, survival, and product quality, posing a threat to the sustainability of the aquaculture industry.

The identification and characterization of fungal species associated with prawns are essential for understanding the diversity of fungal communities, their potential pathogenicity, and the development of effective management strategies. The Olomore market in Abeokuta North Local Government, Ogun State, Nigeria, is a hub for the distribution and sale of various seafood products, including prawns. Investigating the fungal communities present in prawns obtained from this market can provide valuable insights into the potential risks associated with the consumption and handling of these seafood products. This study aims to isolate and characterize the fungi present in Penaeus monodon (giant tiger prawns) obtained from the Olomore market in Abeokuta North Local Government, Ogun State, Nigeria. The findings from this research will contribute to the understanding of the fungal diversity associated with prawns and inform the development of strategies to ensure the safety and quality of seafood products.

## Materials and Methods

Sample Collection
Fresh prawn samples were purchased at Olomore market in Abeokuta, Ogun state. The prawn were aseptically picked at random using sterile gloves and placed into sterilized container, then placed in an iced pack and labelled. They were transported to the microbiology research laboratory of the Crescent University, Abeokuta within one hour of collection for microbiological analyses.

### Sample Preparation
The prawn samples were processed as follows: The gastrointestinal tract, skin, and head of the prawns were removed aseptically using a sterile blade. The remaining prawn meat was crushed into a paste using a sterile mortar and pestle. The skin was swabbed using a sterile swab stick (Ezemba et al., 2020).

### Culture Media Preparation
Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes (Ezemba et al., 2020).

### Fungal Isolation
One gram (1g) of the prawn paste was serially diluted (10^-2, 10^-4, 10^-5, and 10^-6) in sterile distilled water. Aliquots (0.1 mL) of the diluted samples were spread-plated on the prepared PDA plates and incubated at 25°C for 24-48 hours. The growth of fungi on the plates was observed and recorded. Fungal colonies with distinctive characteristics were selectively sub-cultured onto fresh PDA media. This process was repeated until pure colonies were established, ensuring the isolation of specific microbial strains for further analysis (Omoya and Ajayi, 2020).

### Fungal Enumeration
Colony counts from the pure fungal cultures were performed and converted for dilution to express the microbial load in colony-forming units per gram (CFU/g) of the original prawn sample (Peter et al., 2023).

### Molecular Identification of Fungal Isolates
The molecular identification of the fungal isolates was carried out as described by Omoya and Ajayi (2020), which involved the following steps:1. Genomic DNA Extraction,2. Polymerase Chain Reaction (PCR) Amplification and Purification 3. Agarose Gel Electrophoresis 4. Sequencing 5. BLASTn and Phylogenetic Analysis

The obtained DNA sequences were compared with the sequences available in the National Center for Biotechnology Information (NCBI) database using the BLASTn algorithm. Phylogenetic analysis was performed to determine the taxonomic position of the fungal isolates.

**Results**

Table 1: Total Fungal Counts of Prawn Sold in Olomoore Market in Abeokuta

| Sample ID | Total Viable Count (x 10-$^5$ CFU/g) | | |
|---|---|---|---|
| | Gut | Skin | Stomach |
| PPOa | 2 | 4 | 2 |
| PPOb | 2 | 4 | 1 |
| PPOc | NG | NG | NG |
| PPOd | NG | NG | NG |
| PPOe | NG | NG | NG |
| PPOf | NG | NG | NG |

**Key:** NG: No Growth

The table presents the total fungal counts of prawn samples sold in Olomoore Market in Abeokuta. The data is organized by sample ID, with the total viable count (in x 10^-5 CFU/g) reported for the gut, skin, and stomach of each sample. Samples PPOa and PPOb had fungal growth in all three areas (gut, skin, and stomach), with counts ranging from 1 x 10^-5 CFU/g to 4 x 10^-5 CFU/g. Samples PPOc, PPOd, PPOe, and PPOf showed no growth (NG) in any of the three areas, indicating the absence of detectable fungal contamination in these samples.

Overall, the data suggests that some of the prawn samples had fungal contamination, particularly in the gut and skin, while others were free of detectable fungal growth. The variation in fungal counts between samples may be due to differences in handling, storage, or other factors affecting the microbial quality of the prawns.

Table 2: Cultural and Microscopic Characteristics of Fungi Isolated from Prawn

| Isolate ID | Cultural characteristics | Microscopic characteristic | Suspected Organism |
|---|---|---|---|
| PPO1 | Yellowish-Brown, Globose conodia, 5cm, filamentous | Septate hyphae, Globose conidia, loosely radiate vesicle | *Aspergillus spp* |
| PPO2 | Wooly, dark, round sporangia, dominate the whole plate | No septa, sporangiospores are round, colorless Spores produced in large numbers inside the sporangium, Rhizoids like | *Mucor spp* |

The cultural and microscopic characteristics of the two representative fungal isolates, PPO1 and PPO2, are summarized in Table 2. Isolate PPO1 was identified as *Aspergillus* spp., while isolate PPO2 was identified as *Mucor* spp.

**Frequency of Occurrence of Fungi Isolated from Prawn in Olomoore Market**

Table 3 presents a quantitative analysis of the prevalence of fungi in prawn from Olomoore Market. It shows the dominant presence of *Aspergillus spp*., which was found in 12 out of 15 instances (80%) of the occurrences. In contrast, *Mucor spp.* was identified much less frequently, with only 3 occurrences (20%).

**Table 3: Frequency of Occurrence of Fungi Isolated from Prawn in Olomoore Market**

| Fungi ID | Frequency of occurrence | Percentage of occurrence |
|---|---|---|
| *Aspergillus spp* | 12 | 80 |
| *Mucor spp* | 3 | 20 |
| Total | 15 | 100 |

**Table 4: Molecular Identification of the Most Occurred Fungi Isolates**

| Sample ID | Organism | Sequence length (bp) | % Identity | Accession Number |
|---|---|---|---|---|
| PPO | *Aspergillus aculeatus* strain CUAB-OLU | 581 | 99.14% | PP830121 |

The most occurred fungal isolate was identified as *Aspergillus aculeatus* strain CUAB-OLU based on the molecular analysis. The sequence length for the identified isolate is 581 base pairs (bp). The isolate showed a high sequence identity of 99.14% to the reference *Aspergillus aculeatus* strain CUAB-OLU sequence in the database. The accession number for the reference sequence is PP830121, which can be used to retrieve the full sequence information from the relevant database. Overall, the results indicate that the predominant fungal isolate in the sample is closely related to the known species *Aspergillus aculeatus*, with a high degree of sequence similarity to the reference strain. This information can be useful in further characterizing the fungal community composition and potential implications in the studied context.

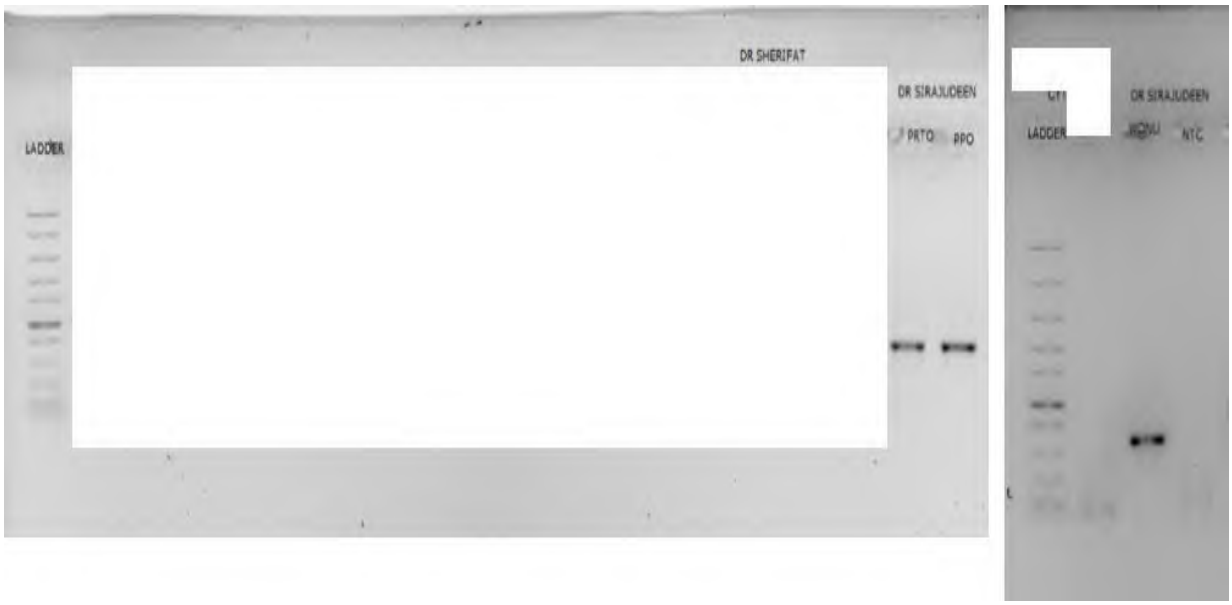


Figure 1: Gel Electrophoresis Result of *Aspergillus aculeatus* strain CUAB-OLU

**Figure 2: Phylogenetic Tree Showing the Relationship of Strain CUAB-OLU with Isolates from NCBI**

Figure 2 offers a detailed representation of the genomic DNA plasmid profiles for the *Aspergillus aculeatus* strain CUAB-OLU. The analysis highlights the plasmid's circular conformation, a characteristic feature of such DNA structures. This particular strain is defined by a genome comprising 583 base pairs, along with seventeen unique restriction sites

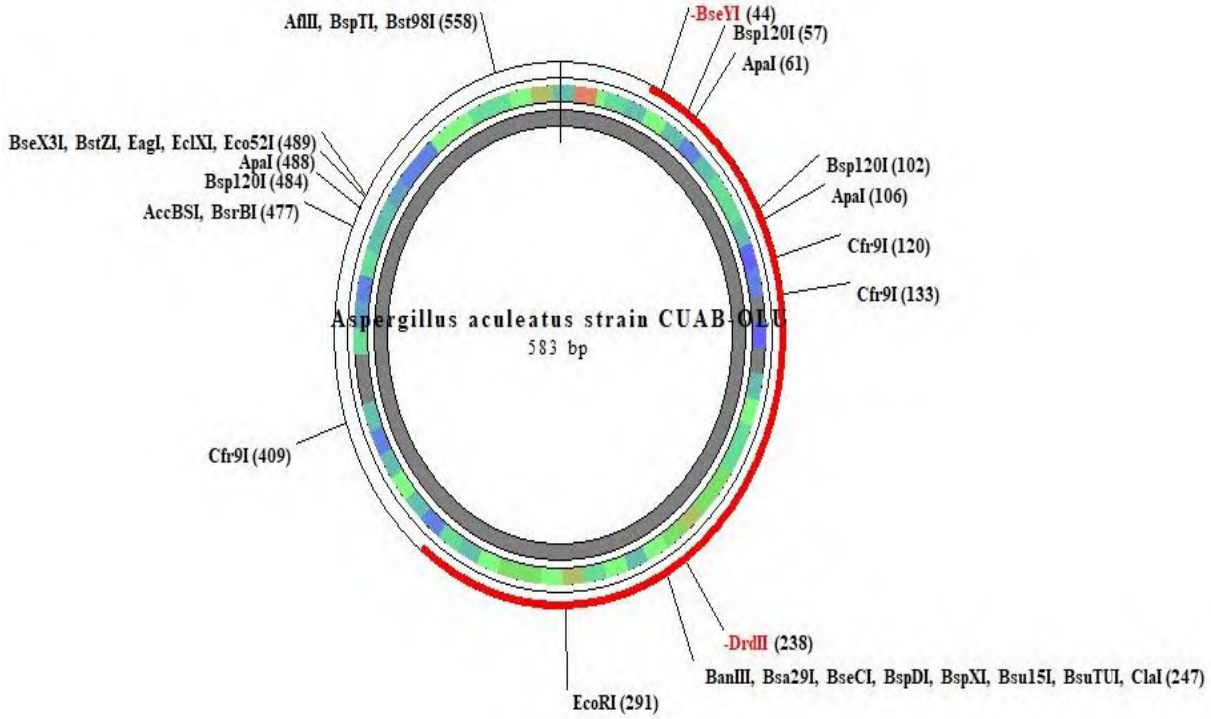


Figure 3: Genomic DNA Plasmid Profiles of *Aspergillus aculeatus* strain CUAB-OLU

**Discussion**

The findings from this study are quite significant in terms of food safety and public health. The presence of viable fungal counts in prawn samples, specifically in the skin with counts as high as 4 x $10^5$ CFU/g, raises concerns about the quality and storage conditions of seafood sold in Olomoore Market, Abeokuta. The lower counts in the head and gut areas, especially with the gut of PPOb showing the lowest at 1 x $10^5$ CFU/g indicated that different parts of the prawn may be susceptible to varying levels of fungal contamination. The absence of fungal growth in samples PPOc through PPOf, indicated is noteworthy. It could point to the possibility of some prawns being less exposed to contamination or being handled in a manner that prevents fungal growth. The counts recorded in this study are lower than 2.07 x $10^6$, 0.39 x $10^6$ and 0.79 x $10^6$ for the head, body and tail of shrimp, respectively reported by Ezemba *et al.* (2020). Similarly, Peter *et al.* (2022) reported a higher fungal count in ready-to-eat (RTE) shrimps in Ibadan. The variation could be as a result of higher levels of fungal contamination that that considered in this study.

The findings regarding the cultural and microscopic characteristics of fungi isolated from prawns are quite revealing. The identification of *Aspergillus species* and *Mucor species* from the samples indicates a diverse fungal population associated with the prawns. The fact that *Aspergillus species* were found in 80% of the samples, while *Mucor species* were present in only 20%, suggests that *Aspergillus* is the more prevalent contaminant in this environment. This predominance of the *Aspergillus* could be due to

its robust nature and ability to thrive in various conditions, which might not be as favourable for *Mucor species*. The high frequency of *Aspergillus sp.* also raises concerns about potential health risks, as some species within this genus are known to produce mycotoxins that can be harmful if ingested. These findings agreed with those of Omoya and Ajayi (2020) and Ezemba *et al.* (2020) who also reported the presence of both *Aspergillus species* and *Mucor species* in shrimps. However, both studies reported the presence of other fungi such as, *Penicillium species, Fusarium species and Rhizopus species* which were not recorded in this study. Moreover, in alignment with this study, Ezemba *et al.* (2020) reported the dominance of *Aspergillus spp* in shrimp but at a lower frequency of 25%. The differences could be because of the variation in the organisms, sources and methods of analysis.

Moreover, the most prevalent isolate, *Aspergillus sp* was specifically identified as *Aspergillus aculeatus* strain CUAB-OLU via the molecular method. The presence of *Aspergillus aculeatus* in the prawn is a matter of concern for food safety and public health. *Aspergillus aculeatus* is a species of fungus that can be found in various environments including marine (Bankole *et al.*, 2022). However, when it comes to food products like prawns, the presence of this fungus could indicate potential spoilage or improper storage conditions (Ogbonna *et al.*, 2022). *Aspergillus* species, including A. aculeatus, are capable of producing mycotoxins, which are toxic compounds that can pose health risks if consumed (Navale *et al.*, 2021). The fact that this particular strain was identified in prawn samples indicated that there may be a risk of mycotoxin contamination, which could lead to foodborne illnesses if the prawns are consumed without proper cooking or handling.

The phylogenetic analysis revealed the genetic relationships and evolutionary history of *Aspergillus aculeatus* strain CUAB-OLU. The fact that this novel strain shows a significant genetic similarity to other *Aspergillus* strains in the NCBI database is indicative of a shared evolutionary path. This connection to established strains provided valuable insights into the genetic makeup and potential characteristics of CUAB-OLU, which can be important for both scientific research and practical applications in fields such as biotechnology and medicine.

The genomic DNA plasmid profiles for *Aspergillus aculeatus* strain CUAB-OLU revealed the fungal strain's genetic architecture. The circular conformation of the plasmid is a key characteristic of prokaryotic DNA, which often carries additional genes that can confer advantages such as antibiotic resistance or metabolic capabilities. The presence of 583 base pairs and seventeen unique restriction sites within this plasmid's genome is particularly noteworthy. These restriction sites are essential for genetic engineering, as they allow scientists to cut and insert specific gene sequences, facilitating the study of gene function and the development of new genetic modifications.

## Conclusion

This study highlights significant food safety and public health concerns related to fungal contamination in prawns. The detection of high fungal counts, particularly *Aspergillus aculeatus* strain CUAB-OLU emphasized the need for improved handling and storage practices to prevent potential health risks associated with mycotoxin production.

**References**

Briggs, M., Funge-Smith, S., Subasinghe, R., & Phillips, M. (2004). Introductions and movement of Penaeus vannamei and Penaeus stylirostris in Asia and the Pacific. FAO Fisheries Technical Paper, 476.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Food and Agriculture Organization of the United Nations, Rome.

Oidtmann, B. (2012). Review of biological factors relevant to import risk assessments for epizootic ulcerative syndrome (Aphanomyces invadans). Transboundary and Emerging Diseases, 59(6), 26-39.

Boenish, R., Kritzer, J. P., Kleisner, K., Steneck, R. S., Werner, K. M., Zhu, W., and Mimikakis, J. (2022). The global rise of crustacean fisheries. *Frontiers in Ecology and the Environment*, *20*(2), 102-110.

Dabadé, D. S., Yessoufou, N., Adido, L., Azokpota, P., and Hounhouigan, D. J. (2023). Quality changes, potential spoilage organisms, and shelf-life prediction of brackish river prawn (*Macrobrachium macrobrachion*) at different storage temperatures. *International Journal of Food Microbiology*, *405*, 110344.

Ezemba, C. C., Mbah, O. G., and Osuala, O. J. (2020). Fungal Quality of Fresh Shrimp sold at Ndibe Beach in Afikpo, Ebonyi State. *International Journal of BioSciences and Technology IJBST*, *13*(4), 42-50.

Fagbohun, E. D., Popoola, O. A., and Durojaiye, A. O. (2021). Mycoflora and Nutritional Analysis of Smoked Dried Crayfish (*Penaeus monodon*–Prawns) During Storage. *Food*, 3, 49 -71.

Omoya, F. O., and Ajayi, A. T. (2020). Assessment of the microbial quality of seafood and effects of salt concentration and temperature on isolated microorganisms. *Journal of Microbiology and Antimicrobials*, *12*(1), 17-31.

Osman, E. O., and Weinnig, A. M. (2022). Microbiomes and obligate symbiosis of deep-sea animals. *Annual review of animal biosciences*, *10*, 151-176.

Peter, B. N., Adedeji, O. B., Okocha, R. C., and Okon, E. M. (2023). Microbial quality of ready-to-eat shrimps from three selected markets in Ibadan. *Journal of Animal Health and Production*, 11(3), 296-305.