Isolation and Characterization of Fungal Strains From Tilapia (*Oreochromis Niloticus*) and Machoiron (*Chrysichthys Nigrodigitatus*) Fish in Taabo Lake, Kossou Lake, and Tagba Lagoon of Grand-Lahou, Côte D’ivoire

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ABSTRACT

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| This study aimed to isolate and characterize fungal strains contaminating freshwater fish (Tilapia and Machoiron) in three water bodies in Côte d'Ivoire: Lake Taabo, Lake Kossou, and the Tagba Lagoon in Grand-Lahou. A total of 120 samples were collected during four seasonal campaigns (dry and rainy seasons) and analyzed in the laboratory. Molds were isolated on acidified Potato Dextrose Agar (PDA) medium (pH 3.5) at 30°C and identified through macroscopic (color, texture) and microscopic (spore morphology, hyphae) observations. Contamination frequencies were calculated for each fungal genus. Among the 156 isolated strains, five dominant genus were identified: *Aspergillus* (57.69%), *Rhizopus* (25%), *Fusarium* (11.54%), *Penicillium* (3.85%), and *Absidia* (1.92%). *Aspergillus* was ubiquitous, while *Rhizopus* and *Fusarium* were more abundant during rainy seasons. The analyzed fish showed high contamination rates: 75% for *Aspergillus*, 32.5% for *Rhizopus*, and 15% for *Fusarium*. These results confirm the presence of potentially toxigenic molds in fish, with risks of mycotoxin production (aflatoxins, ochratoxins). The contamination reflects environmental conditions and local practices. The study recommends establishing regulatory standards to limit consumer exposure and improve food safety. |

*Keywords: Molds, freshwater fish, Côte d’Ivoire, seasonal variation, Tilapia, Machoiron.*

1. INTRODUCTION

Freshwater fish constitute an essential resource for many populations, notably those of Africa. In Côte d’Ivoire, these fishery resources play a crucial role in the diet and economy of riverside populations. Indeed, these fish represent 50% of consumption, which is estimated at 650,000 tonnes in 2021, while providing 42% of animal proteins and genusting more than 70,000 direct jobs and 400,000 indirect jobs (Failler *et al*., 2014; FAO, 2022; Mason *et al*., 2022). However, these fish may contain chemical, physical and biological contaminants capable of causing health disorders affecting the fish and potentially consumers through the production of toxins, then deteriorating their market quality. Indeed, in humid tropical zones, climatic conditions are favorable to the proliferation of microscopic fungi, which most often leads to the contamination of foodstuffs like fish (Chelack *et al*., 1991). The molds can be found in natural environments, including freshwater ecosystems and fish through fish feeding, by runoff from fields contaminated by molds, and by degradation of organic matter. (Caruso *et al*., 2013; Yang *et al*., 2023). The contamination of freshwater fish by molds is therefore a subject of growing concern due to the potential impacts on fish health and, consequently, on human health of consumers. It is within this perspective that the present study is situated, which aims to isolate and characterize the fungal strains responsible for contamination of freshwater fish in order to contribute to the research for bio-indicators of freshwater pollution in humid tropical zones.

2. material and methods

2.1 Material

2.1.1 Biological material

The study was conducted on two species of fresh freshwater fish, Tilapia (*Oreochromis niloticus*) and Machoiron (*Chrysichthys nigrodigitatus*) collected from fishermen of Taabo lake, Kossou lake and Tagba lagoon at Grand-Lahou.



**Fig. 1. Freshwater fish used in this study**

**A**: *Machoiron fish (Chrysichthys nigrodigitatus);* ***B****: Tilapia fish (Oreochromis niloticus)*

2.1.2 Laboratory equipment

In the course of this study, several items of laboratory equipment and consumables were used: Culture medium: Sabouraud with chloramphenicol and PDA, Oven set at 25°C, Stomacher bags, Scalpel blade, Pipettes, Petri dishes, Blades and slides, Methylene blue and Light microscope.

2.2 Methods

2.2.1 Characterization of study areas

The freshwater fish in this study were collected from three (03) rivers: **The Tagba Lagoon in Grand-Lahou**, which originates from the Bandama River, is located in the Grands-Ponts region of southern Côte d'Ivoire. This area experiences a Guinean equatorial climate, characterized by alternating periods of two rainy seasons and two dry seasons of variable duration. Annual average temperatures reach 27.5°C, with average annual rainfall of 1664 mm, concentrated between June and July (Alexandre *et al*., 2019). **Lake Taabo**, also fed by the Bandama River, is located in the Agnéby-Tiassa region. This area features an Attiéen climate with rainfall patterns characteristic of transitional equatorial zones. The region experiences four distinct seasons: two rainy seasons (including a main period from April to June and a shorter one from September to November) and two dry seasons (with a long period from December to March and a short one from July to September) (Koffi *et al*., 2018; Kouassi *et al*., 2007).

**Lake Kossou**, located in the Yamoussoukro District of the Lacs region, is fed by the Bandama River. The area has a well-defined seasonal climate pattern: two dry periods (November-February and July-August) alternate with two rainy seasons (March-June and September-October). Precipitation is more abundant in the eastern sector than the west, peaking in May, June, and September. Temperatures range between 19 and 34°C throughout the year (Groga *et al*., 2022).

2.2.2 Sampling

Across the three (03) study areas, four (04) sampling campaigns were conducted between December 2023 and November 2024, corresponding to the seasonal subdivisions of the study zones: the long dry season (December 2023 to March 2024), the long rainy season (April to July 2024), the short dry season (July to September 2024), and the short rainy season (September to November 2024). During each campaign, ten (10) fish were collected per campaign and per waterbody, totaling one hundred twenty (120) freshwater fish samples (n=120) obtained from fishermen between 8:00 and 10:00 GMT. At each sampling, dissolved oxygen, water temperature, and pH were measured in situ using an HQ40d multi-parameter meter. The samples were placed in a cooler under aseptic conditions at -4°C for transport to the INP-HB Food Microbiology Laboratory for subsequent analysis.

2.2.3 Isolation and Purification of Molds

We isolated molds under aseptic conditions by directly culturing fish skin, flesh, and internal organs on acidified PDA medium (pH 3.5). The medium's pH was adjusted to 3.5 with 10% citric acid to selectively promote mold growth while inhibiting bacteria. For PDA preparation, we mixed 20g potato dextrose powder, 15g agar, and 15g glucose in 1L distilled water, autoclaved at 121°C for 15 minutes, then added the citric acid solution before pouring into 90mm diameter Petri dishes (Emanfo *et al*., 2013). Once the PDA medium solidified, 10g of fish tissue fragments from each sample were directly inoculated onto the agar surface. The cultures were then incubated at 30°C for 3 to 5 days (Abdoullahi *et al*., 2019a). To ensure fungal strain purity, a cloning process was implemented. This process involves performing successive subcultures by collecting a fragment from a fungal colony while avoiding contact with other colonies, then transferring it to fresh culture media to isolate individual colonies (Guiraud, 1998).

2.2.4 Mold Identification

***2.1.1.1 Macroscopic Identification***

Macroscopic identification of strains was performed by visual observation of young fungal cultures (<5 days old) on PDA medium, based on cultural characteristics described by Pitt & Hocking (1997, 2009); Samson *et al*. (2014). The observed cultural characteristics related to the fungus's genusl appearance included: mycelium color, shape, and texture; pigment production; spore density and features; and the fungal growth rate on the culture medium. Regarding surface morphology, strains exhibited fluffy, velvety, powdery, granular, flat, or raised appearances (Compaore *et al*., 2016; Olga *et al*., 2015).

***2.1.1.1 Microscopic Identification***

For microscopic identification of fungal strains, fresh mount preparations were made following the protocols described by Abdoullahi et al. (2019) ; Nguyen (2007). A small quantity of fungal culture was collected using a sterile needle and streaked onto a glass slide. After application of 1% methylene blue, the fresh smear was examined under a microscope at ×40 and ×100 magnifications. Microscopic analysis focused on evaluating the following characteristics: Morphology and branching patterns of mycelial filaments, conidiophore structure, presence and morphology of vesicles, conidiogenous cell arrangement (uniseriate or biseriate), spore features: size, shape, coloration, and surface ornamentation (verrucose, smooth, or granular) (Abarca *et al*., 2004; Boudih, 2011; Compaore et al., 2016; J. I. Pitt & Hocking, 1997, 2009a; Schuster *et al*., 2002). The prevalence of fungal genus contaminating freshwater fish and the contamination frequency of collected fish by specific genus across different locations were calculated using the Marasas *et al*. (1988).

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3. results and discussion

3.1 Results

3.1.1 Characterization of Isolated Fungal Strains

The isolation procedure performed on fresh Tilapia and Machoiron fish from Lake Taabo and Tagba Lagoon in Grand-Lahou yielded 52 fungal strains. The cultural and microscopic characteristics of representative fungal genus are documented in tables 1 and 2, respectively.

3.1.2 Identification of Isolated Fungal Strains

One hundred fifty-six (156) mold strains were isolated from fresh Machoiron and Tilapia fish collected in Taabo Lake, Kossou Lake, and Tagba Lagoon of Grand-Lahou across four climatic seasons (Table 3). These strains were classified into 5 genus: *Aspergillus* section *Nigri* (Moi 09) and *Flavi* (Moi 21), *Fusarium* (M4-B3), *Absidia* (M1-B3), *Penicillium* (H2O1), and Rhizopus (T-B1).

The *Aspergillus* genus was dominant with ninety (90) strains, representing 57.69% of all isolated fungal strains. Thirty-nine (39) strains belonged to *Rhizopus* (25%), while eighteen (18) *Fusarium* strains were obtained (11.54%). Finally, six (06) *Penicillium* strains and three (03) *Absidia* strains were isolated at respective proportions of 3.85% and 1.92%.

Regarding *Aspergillus*, 69 of the 90 isolated strains belonged to *Aspergillus* section *Nigri* (76.67%). The remaining 23.33% comprised *Aspergillus* section *Flavi* (16.67%) and *Aspergillus section Fumigati* (6.66%). Figure 2 below shows the prevalence of different fungal genus isolated from fish.

**Table 1. Macroscopic characteristics of fungal strains isolated from fresh fish**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Codes** | **PDA Culture Description** | **Macroscopic Images** | | **Reference Images** |
| **Obverse** | **Reverse** |
| Moi 21 | Rapid growth with rounded margins, compact thalli initially yellow becoming olive-green at maturity with fluffy texture; red pigment production on colony reverse. |  |  | A. flavus isolated from aural debris  **(Pitt & Hocking, 1997)** |
| T-B1 | Highly invasive, filamentous strain displaying white to gray coloration with black sporangia at margins; culture reverse appears colorless. |  |  | **(BEUGRE, 2024)** |
| H201 | Rapid growth with velvety appearance, white thallus and dark green conidia; red pigments visible on colony reverse. |  |  | **(BEUGRE, 2024)** |
| Moi 09 | Very rapid growth with rounded margins, extremely compact black thalli displaying granular texture; no diffusible pigments observed on colony reverse. |  |  | **(Pitt & Hocking, 1997)** |
| M4-B3 | Rapid growth with aerial mycelia and crescent-shaped, septate conidia of cream-white coloration; pink to red pigments visible on colony reverse. |  |  | **(Chabasse et al., 2002)** |
| M1-B3 | Rapid growth covering the Petri dish, forming low white colonies with granular texture; beige toyellow pigments visible on colony reverse. |  |  | **(Chabasse et al., 2002)** |

**Table 2. Microscopic characteristics of fungal strains isolated from fresh fish**

|  |  |  |  |
| --- | --- | --- | --- |
| **Codes** | **Microscopic Description** | **Microscopic Images** | **Reference Images** |
| Moi 21 | Septate and branched hyphae with rough-walled conidiophores. Vesicles bearing biseriate phialides and metulae are arranged radially, forming conidial heads containing oval, green-colored spores. |  | **(Pitt & Hocking, 2009b)** |
| T-B1 | Nonseptate (coenocytic) and branched hyphae bearing terminal vesicles. Endogenous conidia occur in clusters, displaying oval morphology and gray coloration. |  | **(Pitt & Hocking, 2009b)** |
| H201 | Penicillium-like branched conidiophore with terminal ends containing sparse, oval-shaped spores having smooth walls. Hyphae are intertwined and septate. |  | **(Pitt & Hocking, 2009b)** |
| Moi 09 | Presence of erect conidiophores terminating in circular aspergillate heads with biseriate arrangement (phialides and metulae), producing round, verrucose, black-colored spores**.** |  | **(Pitt & Hocking, 2009b)** |
| M4-B3 | Hyaline, septate hyphae with branched and clustered conidiophores. Conidia are ovoid-elongate, bean-shaped. |  | **(Pitt & Hocking, 2009b)** |
| M1-B3 | Broad, aseptate hyphae. Aerial mycelia forming globose, flattened sporangia. |  | Voir les détails de l’image associée. ATLAS MICOLOGIA: ABSIDIA CORYMBIFERA / Lichtheimia corymbifera  **(Pitt & Hocking, 2009b)** |

**Fig. 2. Prevalence of different fungal genus isolated from fish**

The *Aspergillus* genus, contaminating 75% of analyzed fish, was present across all sites (Lake Taabo, Tagba Lagoon, and Lake Kossou) and all seasons. The *Rhizopus* genus, contaminating 32.5% of fish, was predominantly observed during rainy periods. *Fusarium* contaminated 15% of fish, particularly during dry-to-rainy transition seasons. Finally, *Penicillium* (5%) and Absidia (2.5%) showed contamination primarily during rainfall events.

Table 3 below presents the contamination frequency of collected fish by detected genus across different locations.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Fungal genera** | **Localities** | | | | | | | | | | | | **Number of contaminated fish** | **Frequency of genus contamination of samples** |
| **Lake of Taabo** | | | | **Tagba Lagoon in Grand-Lahou** | | | | **Lake of Kossou** | | | |
| LDS | LRS | SRS | SDS | LDS | LRS | SRS | SDS | LDS | LRS | SRS | SDS |
| *Aspergillus* | + | + | + | + | + | + | + | + | + | + | + | + | 90 (120) | 75 % |
| *Rhizopus* | - | + | - | + | - | + | + | + | + | + | - | + | 39 (120) | 32,5 % |
| *Fusarium* | + | + | - | + | - | - | + | + | + | + | - | + | 18 (120) | 15 % |
| *Penicillium* | - | + | - | - | - | + | - | - | - | + | - | + | 6 (120) | 5 % |
| *Absidia* | - | + | - | - | - | + | - | - | - | + | - | - | 3 (120) | 2,5 % |

*\*The numbers in parentheses represent the total sample count; +: Mold presence; -: Mold absence; LDS: Long Dry Season; LRS: Long Rainy Season; SRS: Short Rainy Season; SDS: Short Dry Season.*

3.1 Discussion

This study investigated the presence of toxigenic molds in freshwater fish from Lake Taabo and Tagba Lagoon in Grand-Lahou. One hundred fifty-six (156) strains were identified, belonging to 5 fungal genus*: Aspergillus, Rhizopus, Fusarium, Absidia*, and *Penicillium*. The isolation of *Penicillium*, *Fusarium*, and *Aspergillus* in this study is supported by the work of Bashorun *et al*. (2023) on aquaculture fish tissues and feed in Doha, Qatar, which identified strains of *Aspergillus, Penicillium*, and *Fusarium*. Similarly, studies on round fish and their feed from 15 fish farms in São Paulo and Dourados, Brazil ; Nile tilapia (*Oreochromis niloticus*) from Giza markets, Egypt ; Nile tilapia and fish feed from Egyptian aquaculture farms and rainbow trout (*Oncorhynchus mykiss*) feed in Iran have also isolated fungal strains of *Aspergillus, Penicillium, Rhizopus, Fusarium*, and *Absidia* (Alinezhad et al., 2011; Anees et al., 2023; Gomes et al., 2022; Mohamed et al., 2017).

Similarly, other studies have reported comparable findings regarding genus frequencies, notably *Aspergillus* (75%), *Rhizopus* (32.5%), *Fusarium* (15%), *Penicillium* (5%), and *Absidia* (2.5%). These results align with those of Alinezhad et al. (2011) ; Mohamed et al. (2017) who predominantly isolated *Aspergillus* at respective frequencies of 57% and 64%. Conversely, Gomes et al. (2022), found *Penicillium* as the second most common genus (12.84%), followed by *Rhizopus* (11.54%), *Absidia* (11.01%), and *Fusarium* (3.84%). The predominance of *Aspergillus* in analyzed samples may be explained by its ubiquity - present in soil, air, and water as spores. According to Pitt & Hocking, (2009), *Aspergillus* species, belonging to the *Ascomycota subphylum*, are capable of sexual reproduction and readily colonize food products when conditions are favorable.

According to Luan et al. (2023), freshwater fish contamination by molds in their natural environment depends on their feeding habits and environmental factors such as geographic location, humidity, temperature, and hygiene conditions. Indeed, Liu et al. (2016) analyzed the gut contents of eight wild fish species from the same lake and found significantly different microbial communities between carnivorous and herbivorous fish species.

Furthermore, studies conducted in fish farms on fish feed and fish tissues have shown a direct correlation between fungal contamination of fish feed and contamination of the fish tissues themselves (Bashorun et al., 2023; Gomes et al., 2022). Moreover, surface water pollution, exacerbated by industrial, agricultural, and domestic discharges, promotes the proliferation of fungal strains (Vieira et al., 2023). Specifically, *Penicillium* implicatum - a citrinin-producing fungus of the *Penicillium* genus - contributes to organic matter decomposition in fish ponds, leading to contamination (Damasceno et al., 2019). Chronic exposure of aquatic species to water contaminated with mycotoxigenic fungi may cause pathologies in fish and pose health risks to consumers.

In Côte d'Ivoire, a humid tropical region, the presence of these microorganisms reflects widespread contamination of freshwater fish, with potential mycotoxin production (including ochratoxin, aflatoxin, and fumonisin) in fish flesh.

4. Conclusion

In conclusion, this study reveals that fish from Lake Taabo and Tagba Lagoon (Grand-Lahou) harbor significant fungal diversity, including species potentially pathogenic to humans due to their toxin-producing capacity. Analysis of isolated fungi showed marked predominance of Aspergillus molds, followed by *Rhizopus, Fusarium, Absidia*, and *Penicillium* genera. The mycotoxins produced by these strains cause substantial economic losses through disease and mortality in contaminated fish. Furthermore, even at low concentrations, these mycotoxins pose serious health risks to fish consumers. To ensure food safety and implement effective freshwater

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