

## Original Research Article

# ISOLATION AND IDENTIFICATION OF FUNGAL STRAINS FROM FRESH AND SMOKED FISH FROM THE SASSANDRA RIVER IN CÔTE D'IVOIRE

### ABSTRACT

**Introduction:** Fresh and smoked fish are widely consumed in Côte d'Ivoire as everywhere in West Africa. However, these foodstuffs, due to certain processing conditions, are likely to be contaminated by molds that produce dangerous mycotoxins.

**Objective:** This study aimed to isolate and identify fungal strains contaminating fresh and smoked fish from the Sassandra River in Côte d'Ivoire.

**Place and date of the study:** Sampling was carried out in various processing sites around the Sassandra River, particularly in the towns of Soubré and Guessabo. The microbiological analysis was carried out at Jean LorougnonGuédé University in Daloa (Ivory Coast).

**Methods:** A total of 108 samples of fresh and smoked fish were collected. Isolation and purification of fungal strains were carried out on Sabouraud medium with chloramphenicol. The identification of isolated strains was made on the basis of morphological and cultural criteria.

**Results:** A total of 126 fungal strains were isolated, including 87 from the samples from Guessabo and 39 from those from Soubré. The predominant species were *Aspergillus* of the *Glaucus* group (39%), *Aspergillus niger* (36%) and *Penicillium* sp. (25%).

**Conclusion:** This study shows that fresh and smoked fish from the Sassandra River in Côte d'Ivoire are contaminated by several strains of molds, some of which produce mycotoxins that can cause illness in consumers depending on their concentrations. It would therefore be appropriate to improve processing techniques.

**Comment [u1]:** Specify te quntity from fresh and the quantity from dry fish

*Keywords: fresh and smoked fish, moulds, Sassandra River, Ivory Coast.*

## 1. INTRODUCTION

Fishing and fish processing in Côte d'Ivoire are ancient activities that have great economic, social and cultural importance, since fish covers more than half of the population's protein needs [1]. Fresh fish, even just caught, are not free from contamination because the waters and our entire environment are contaminated by chemical, physical and biological agents that are likely to harm human health. As for the processing of fish, one of the objectives of which is to reduce post-harvest losses and ensure the food security of populations, does not always guarantee the quality of the fish [2; 3]. It is the source of certain harmful effects on the health of processors and consumers as well as on the environment [4; 5; 6]. But being a very perishable commodity because of its very high protein and water content, fish is subject to enormous post-harvest losses due to mold [7]. Molds or filamentous fungi are important

players in the microbial world. They can be defined as filamentous and immobile heterotrophic microorganisms, whose cellular structure is that of a classic eukaryotic cell. Molds, classified among the important germs in food microbiology, are agents of food spoilage, especially fish. The mycotoxins they excrete and present in food are of growing concern [8]. It is within this framework that the present study falls, the general objective of which is to search for the presence of fungal strains in fresh and smoked fish. Specifically, it involves the isolation and identification of fungal strains from fresh and smoked fish from the Sassandra River.

Comment [u2]: Why you choose this zone

## 2. MATERIAL AND METHODS

### 2.1. Sampling

In the towns of Soubré and Guessabo drained by the Sassandra River, one hundred and eight (108) samples of fresh and processed (smoked/dried) fish were randomly collected from different fishermen and processors. The collection of the samples took place from October 15 to November 22, 2022. These samples made up of three species (Tilapia, Mâchoron and Labéo) were taken under aseptic conditions, then transported to the laboratory and kept at 4°C for the time of analyzes.

Comment [u3]: Specify the quantity of fresh and quantity of dry

### 2.2. Mold isolation

From the different batches of samples, a series of decimal dilutions were carried out for each sample. Only the dilutions of 10-1, 10-2 and 10-3 were retained. Three Petri dishes per dilution containing the Sabouraud medium with chloramphenicol were inoculated and incubated at 30° C. for 72 hours. The colonies characteristic of the molds that appeared were isolated and kept for identification.

### 2.3. Subculturing and purification

Previously isolated colonies were subcultured successively until pure strains were obtained, on each Petri dish of a single colony of a fungus [2]. Subculturing was done by taking a fragment of the colony using a sterilized pipette while avoiding its contact with the other neighboring colonies of the same dish on the Sabouraud medium with chloramphenicol [9]. This fragment was placed in the center of a new carefully labeled Petri dish. The pure strains obtained were then stored on Czapek Yeast Extrat Agar medium slanted in cryovials at +4°C [10].

### 2.4. Identification

The identification was made according to the morphological and cultural criteria of the different strains. All the pure strains obtained were subjected to a morphological identification carried out by macroscopic and microscopic observation [11].

#### 2.4.1. Macroscopic identification

Macroscopic identification is done with the naked eye, it is based mainly on the characters such as: the texture of the thallus (velvety, woolly, powdery, cottony, flaky, etc.), the growth speed, the appearance of the airborne mycelium, the color (face and back) colony, odour, thallus color and growth contour [9; 12].

### 2.4.2. Microscopic identification

Microscopic identification was made by microscopic observation using a fragment of the pure strain (a few spores and a mycelial fragment at the margin of the thallus) taken with a sterile platinum loop. This fragment was then transferred to a slide, on which lactophenol-blue was added as a diluent. Microscopic observation was made at magnifications 10 and 40 [13; 14; 15].

## 3. RESULTS AND DISCUSSION

### 3.1. Results

#### 3.1.1. Characteristics of isolated strains

The isolation carried out from fresh and processed fish (dried/smoked) made it possible to obtain a good number of fungal strains (126), of which 27 come from fresh fish and 99 from processed fish. 87 of the 126 isolates come from fish sampled in the locality of Guessabo, i.e. 64.05%. The diversity of the strains isolated is contained in the table below (Table 1).

**Table 1:** Diversity of fungal strains isolated from fresh and processed fish from the Sassandra River

GENUS	PHYLUM	CLASS	ORDER	FAMILY
<i>Aspergillus niger</i>	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
<i>Aspergillus</i> of <i>Glaucus</i> group	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
<i>Penicillium</i>	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)

Comment [u4]: Need to separate result of fresh and dry fish

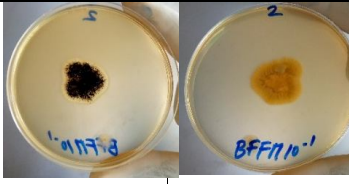
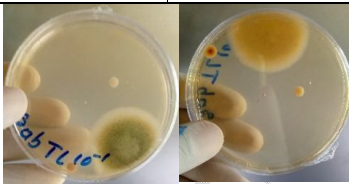

#### 3.1.2. Identification of mold strains

Identification based essentially on the morphological study of mycelium made it possible to identify the following fungal strains: *Aspergillus* of the *Glaucus* group, *Aspergillus niger* and *Penicillium* sp. with a predominance for *Aspergillus* of the *Glaucus* group 39%.

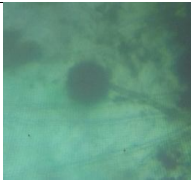
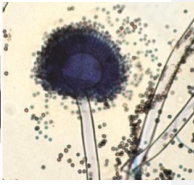

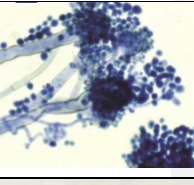
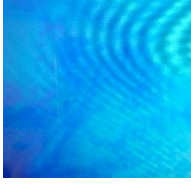
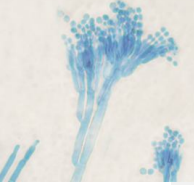
The cultural, macroscopic and microscopic characteristics of the isolated fungal strains are summarized in the tables below (Table 2 and 3).

**Table 2:** Macroscopic characteristics of fungal strains isolated from fresh and processed fish from the Sassandra River.

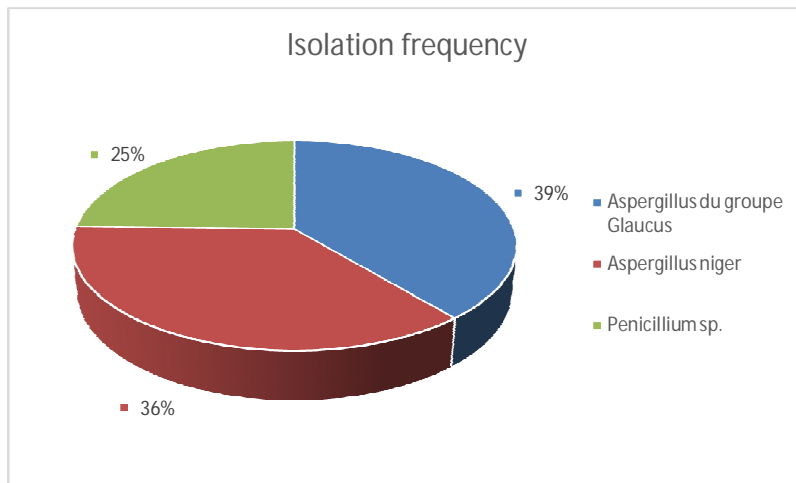
Genres	Description	Straight	Verse
--------	-------------	----------	-------

<i>Aspergillus niger</i>	Colonies first white, then yellow and finally black grainy on the front. On the reverse side, the colonies are colorless to pale yellow.	
<i>Aspergillus</i> of <i>Glaucus</i> group	Front: small colonies, flat, powdery, green in color. Back: orange-yellow to dark brown. Rapid growth.	
<i>Penicillium</i>	The colony is usually fluffy, powdery, variable in color, most often green. The reverse side is colorless or dark.	

**Table 3:** Microscopic characteristics of fungal strains isolated from fresh and processed fish from the Sassandra River.

Genres	Description	microscopic appearance	reference photo
<i>Aspergillus niger</i>	The conidiophore is smooth, hyaline or brownish in its upper half, very long (1.5 to 3 mm).		
<i>Aspergillus</i> of <i>Glaucus</i> group	Conidiophore smooth and colorless; round or club-shaped vesicle; phialides short, stubby, directly inserted on the vesicle.		
<i>Penicillium</i> sp.	The septate, hyaline hyphae carry simple or branched conidiophores, sometimes grouped in a bush or coremia.		

The isolation frequencies of the fungal strains are shown in the figure below (figure 1).



**Figure 1:** Frequencies of fungal species isolated from fresh and processed fish from the Sassandra River.

**Comment [u5]:** Make statistical analysis of the result

**Comment [u6]:** The result of fresh and fish need to be separated

### 3.2. Discussion

Isolation from fresh and processed fish from the Sassandra River revealed 126 fungal strains belonging to 3 genera: *Aspergillus niger*, *Aspergillus* of the Glaucus group and *Penicillium* sp. These fungal genera are generally found in the majority of poorly preserved or insufficiently dried dry foods. Of all the samples analyzed, the genus *Aspergillus* (from the Glaucus (39%) and *Niger* (36%) group) stood out with the highest frequency of occurrence. It is a very common fungus, in the soil and in the air through its spores. The treatment and processing of fish is most often carried out in conditions and places where the humidity level is relatively high, favoring the growth of *Aspergillus*. This could be the reason for the strong presence of this genre. During fieldwork, we witnessed poor storage and packaging conditions for processed fish characterized by exposure of products to the open air and unprotected against dust and flies. These results are similar to those of Rebbouh and Laaid et al [16] who isolated the genus *Aspergillus* mainly with respective frequencies of 37.5% and 85%. The fungi of the genus *Aspergillus* belonging to the subphylum Ascomycotina have a sexual mode of reproduction. Thus, they easily colonize food products when storage conditions are not suitable [17]. To this could be added the neglect of good manufacturing and hygiene practices by fish processors. All these practices are undoubtedly causes of fish contamination which have negative consequences on the health of consumers. These results are in agreement with previous work by Abdoullahi et al., [1] who had isolated the genus *Aspergillus* with a frequency of 45% of transformed fish. It should also be noted that relative humidity and pH are very important parameters which condition the start of fungal growth [18; 19]. Biochemical and physical changes such as oxidation and reabsorption of water by fish, as well as microbiological changes occur during storage of processed fish [20]. Several other studies have obtained a predominance of the genus *Aspergillus* in the contaminating flora of processed fish [20].

Ochratoxin A and patulin are produced by several species of *Aspergillus* and *Penicillium*. Contamination with these mycotoxins is common in foods [21]. In humans the clear evidence

for the association between renal toxicity, kidney cancer and ochratoxin A has not been clearly established although effects on the kidney have been demonstrated. As for patulin, it has been reported in humans to cause nausea, digestive upset and vomiting. Imane and Mouhamed (2012) had isolated fungal strains identical to those in this study, which produce Type B1, B2, G1 and G2 aflatoxins, toxins that could cause liver cancer [22]. The presence of these types of germs gives an idea of the overall contamination of processed fish in localities around the Sassandra River (Côte d'Ivoire). These germs could be the cause of the production of toxins in the fish. Sun drying as well as exposure of processed fish to the open air could explain the high presence of fungal strains.

#### 4. CONCLUSION

It should be noted that fresh and processed fish from the Sassandra River draining the localities of Guessabo and Soubré contain a wide diversity of fungal species, including species incriminated in human pathology. In addition, the analysis of the nature and the frequency of isolation of the fungi shows a clear predominance of molds of the genus *Aspergillus*, in particular *Aspergillus niger*, *Aspergillus* of the *Glaucus* group. The regular presence of fungi in fresh and processed fish calls for adaptation and mastery of processing techniques and good hygiene and manufacturing practices in order to reduce contamination and preserve consumer health.

#### REFERENCES

1. Abdoullahi H.O., Tapsoba F., Guira F., Zongo C., Abakar L.I., Tidjani A. & Savadogo A. (2018). Technologies, quality and socio-economic importance of dried fish in Africa. *Rev. Science. Technol., Synthesis* 37: 49-63p.
2. Depo, A.A., Dossou, J. & Anihouvi, V. (2019). Technical itinerary and quality assessment of catfish (*Clarias gariepinus*) smoked and marketed in Benin. *Earth Life Sciences and Agronomy*, 7: 29-34.
3. Tamgno B.R., Ngunte T.H., Tchatcho N.L.N., Mouamfon M. and Tinkeu N.L.S. (2020). Insect pests of smoked fish during storage and damage caused in the northern loop of the Dja Biosphere Reserve (East Cameroon). *International Journal of Biological and Chemical Sciences*. 14(2): 528-538. Available online at <http://www.ifgdg.org>
4. Djessouho, D.O.C. (2015). Socio-economic analysis of the smoking of fish from artisanal maritime fishing on the coast of Benin. Master's dissertation from the Higher Institute of Agronomic, Agrifood, Horticultural and Landscape Sciences, Agro Campus Ouest (Renne), 56p.
5. Bhat R.V., and Vasanthi S. (2003). Mycotoxin food safety risks in developing countries. *Food Safety in Food Security and Food Trade. Vision 2020 for Food, Agriculture and Environment, Focus* 10, brief.
6. Sameza M.L., Tchameni S.N., Ekoue J.D.A., Jazet P.M.D., Tchoumboungang F. (2016). Growth inhibition of the stored fish (*Ethmalosafrimbata*) fungus *Aspergillus flavus*, exposed to extracted essential oils from *Callistemon citrinus* and *Ocimum canum*. *Af. J. Microbiol. Res.*, 10 (30): 1164-1172. DOI: 10.5897/AJMR2016.8028.

Comment [u7]: Reference need to improved

7. Garrigos, Antonio Roldan. (2016). Contribucion al conocimiento de los hongos acuaticos en el sureste español. *Eubacteria* 36:9.
8. Ndrianaivo E.N., Cornet J., Cardi M., Razanamparany L., Berge J.P. (2016). Storage of smoked and/or dried fish: case of *Oreochromis niloticus* "Fihasalay" from Madagascar. *Africa Sciences*, 12(2): 254–265. DOI: <http://www.africquescience.info>.
9. Pereira E., Santos A., Reis F., Tavares R.M., Baptista P., Lino-Neto T. and Almeida-Aguiar C. (2013). A new effective assay to detect antimicrobial activity of filamentous fungi. *Microbiological Research*. 168 (1): 1-5.
10. Giraud J. (2011). *Food microbiology*. Edition Donod, Paris Mehravar M. & Sardari S;
11. Botton B., Breton A., Fevre M., Gauthier S., Guy P., Larpent J.P., Reymond P., Sanglier J.J., Vayssier Y., and Veau P. (1999). *Useful and harmful molds. Industrial importance*. Mason. Paris.
12. Tabuc C. (2007). *Fungal flora of different substrates and optimal conditions for the production of mycotoxins*. Pathology and Genetics. University of Bucharest.
13. Abdelkader F. (2012). *Comparative study of soil infection by some pathogenic fungi under conventional tillage conditions*. Thesis of Magister, Ferhat Abbas Setif University.
14. Chabasse D, Bouchara JP, De Gentile L, Brun S, Cimon B, Penn P. (2002). *Molds of medical interest*. Training notebook No. 25, Bioforma 230 bd raspail 75014, Paris.
15. Bush EA, Stromberg EL, Hong C, Richardson PA, Kong P. (2006). *Illustration of key morphological characteristics of Phytophthora species identified in Virginia nursery irrigation water*. Online. *Plant Health Progress* doi: 10.1094/PHP-2006-0621-01-RS.
16. Rebbouh S. (2016). *Isolation and identification of fungi associated with the white cochineal parlatoriablanchari (BLANCHARD, 1868) on some varieties of dates*. Master's thesis, KasdiMerbah Ouargla University,
17. Laaid D., Zakaria B., Nouraddine K., and Abdelhakim A.W. (2009), *Effect of secondary metabolites (mycotoxins) of Aspergillus fres. On germination in certain varieties of Chinese peas*, *Journal of Agricultural Sciences of the University of Damascus*. 25(1): 95-106.
18. Hocking A.D. (2006). *Aspergillus and related teleomorphs*. *Food Science*. 451-487.
19. Abdoullahi H.O., Zongo C., Tapsoba F., Tidjani A., Savadogo A. (2016). *Evaluation of the hygienic quality and physicochemical parameters of dried fish sold in the cities of N'djamena (Chad) and Ouagadougou (Burkina Faso)*. *Journal of industrial, health and environmental microbiology*. 10 (1): 13-32.
20. Elia N., Ndrianaivo L., Berge J.P., Cornet J., and Cardinal M. (2016). *Storage of smoked and/or dried fish: case of Oreochromis niloticus "Fihasalay" from Madagascar*. *Africa Science*. 12 (2): 254-265.

21. Riba A., Sabaou N., Mathieu F., and Lebrihi A. (2005). First investigations on fungi producing Ochratoxin A in the cereal sector in Algeria. Euro-Maghreb Symposium on chemical biological contaminants and food safety, Fez.
22. Imane K., and Mouhamed S. (2012). Isolation of some fungi that pollute the irrigation water of the Faculty of Agriculture of the University of Baghdad, Iraqi Journal of Agricultural Science. 43(2): 76-84.

UNDER PEER REVIEW