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 Lorougnon Guédé 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 Guessabo samples and 39 from the Soubré samples from 54 fresh fish and 54 smoked fish.. 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.

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 isolating and identifying fungal strains from fresh and smoked fish from the Sassandra River, chosen for its important role in supplying local markets with freshwater fish..

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 (54) and processed smoked (54) 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 directly from fishermen (for fresh fish) and from processors (for smoked fish), then transported to the laboratory in coolers containing iced and stored at 4°C for the duration of the analyses.

2.2. Mold isolation

From the different batches of samples, a series of decimal dilutions were carried out for each of the 6 batches of samples. Only the dilutions of 10^{-1}, 10^{-2} and 10^{-3} were retained. Three Petri dishes per dilution containing Sabouraud medium with chloramphenicol were inoculated before being incubated at 30°C for 72 hours. After this incubation time, the mushrooms that appeared were subcultured onto a new Sabouraud medium with chloramphenicol in Petri dishes. The re-isolated fungi were purified by successive subcultures. A mycelial fragment approximately 1 cm in diameter was taken from the growth front of the culture and transferred to Petri dishes on Sabouraud medium with fresh chloramphenicol until pure strains were obtained (Giraud et al., 2011). The colonies (pure strains) characteristic of the molds obtained were then 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
From fresh fish				
Aspergillus niger	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
Aspergillus of *Glaucus* group	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
Penicilium	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
From processed (smoked fish)				
Aspergillus niger	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
Aspergillus of *Glaucus* group	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae (hyalohyphomycetes)
Penicilium	Deuteromycotina	Hyphomycetes	Moniliales	Moniliaceae

				(hyalohyphomycetes)

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.

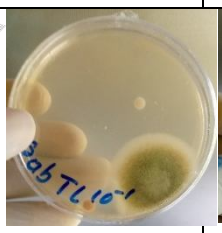
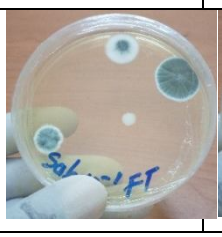
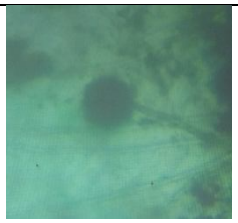
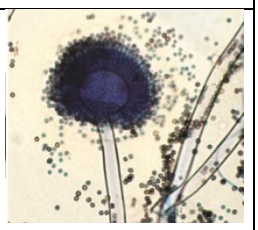
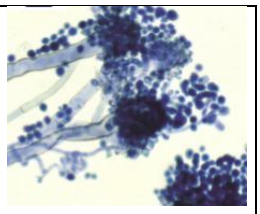
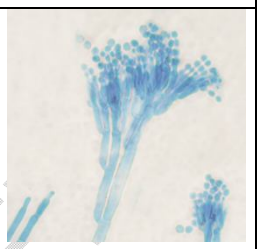
Genres	Description	Straight	Verse
Aspergillus niger	Colonies first white, then yellow and finally black grainy on the front. On the reverse side, the colonies are colorless to pale yellow.		
Aspergillus of *Glaucus* group	Front: small colonies, flat, powdery, green in color. Back: orange-yellow to dark brown. Rapid growth.		
Penicillium	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
Aspergillus niger	The conidiophore is smooth, hyaline or brownish in its upper half, very long (1.5 to 3 mm).		

Aspergillus of *Glaucus* group	Conidiophore smooth and colorless; round or club-shaped vesicle; phialides short, stubby, directly inserted on the vesicle.		
Penicillium 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).

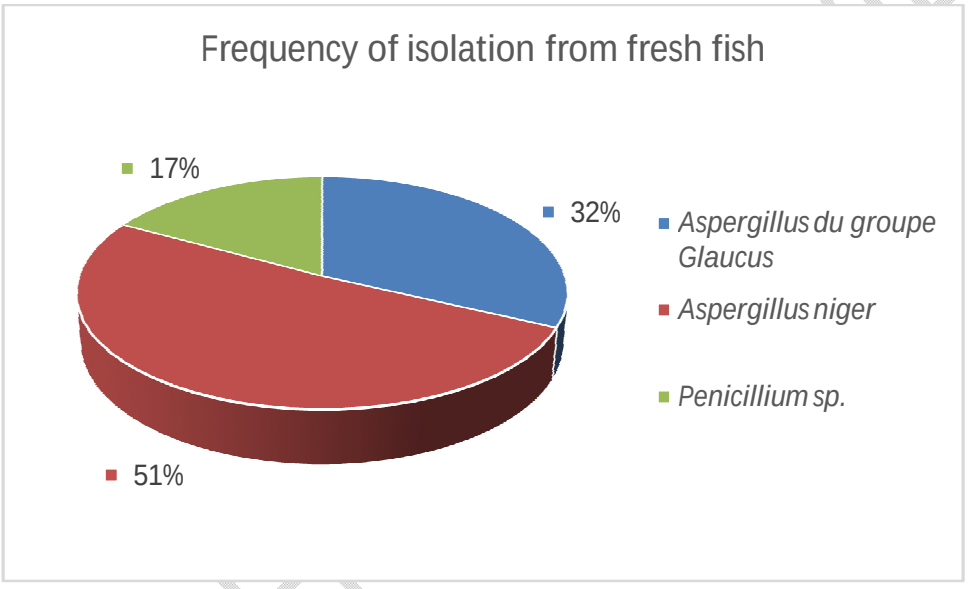


Figure 1: Frequencies of fungal species isolated from fresh fish from the Sassandra River.

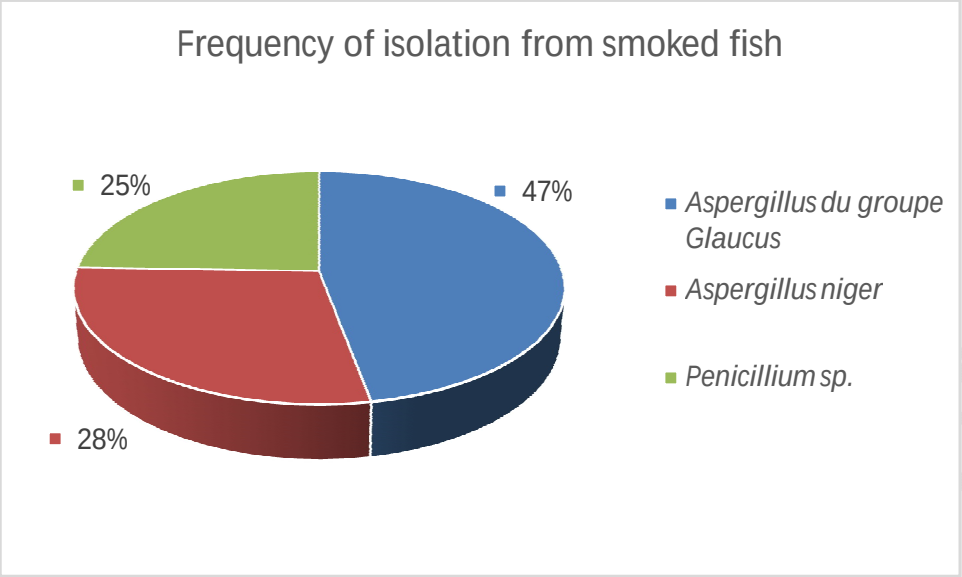


Figure 2: Frequencies of fungal species isolated from smoked fish from the Sassandra River

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.

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