ISOLATION AND IDENTIFICATION OF FUNGI FROM DRIED FISH AND OTHER SEAFOODS SOLD IN LOCAL MARKET AND SELECTED SUPERMARKET IN BATANGAS CITY

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ABSTRACT

Dried fish and other variety of seafood are highly consumed due to its low cost and high availability. It is important to know the quality of the product because continuous consumption of contaminated dried products may pose a health risk to the consumers. 10 grams of each fish species, tuyo, dilis, sapsap, daing, and pusit were obtained. They were randomly selected and thoroughly examined for fungi contamination. Isolation of fungi was carried out by performing washing method, incubating in Sabouraud Dextrose Agar in room temperature for 7 days, and identifying the fungi macroscopically with its morphological structure and with Lactophenol Cotton Blue stain. Fungal growth were described based on color, margin, elevation, and form. Based on the gathered data, the colony of fungi that had been isolated from the Supermarket and Old Market were described in terms of appearance (fuzzy, thready or cottony) and as mold or yeast. Microscopic analysis was done to visualize the characteristic appearance of fungi based on the hyphae, whether aseptate or septate; spore formation, if sporangiospore, arthrospore, conidia, or microconidia; and type of reproduction as to sexual or asexual. This study proved that fungi were present from dried fish and other seafoods sold from Old Market and selected supermarket (Citimart) in Batangas City. Since the presence of these fungal contaminants are noted, eating such products, whether they came from the supermarket or wetmarket, are not safe and harmful to the consumer.

Keywords: *Sabouraud Dextrose Agar, mycotoxins, molds, yeasts*

INTRODUCTION

Fish, a good alternative to meat, is one of the most important sources of protein. It has essential nutrients - high quality protein, unsaturated essential fatty acids, minerals, and water soluble and fat soluble vitamins, to maintain good health (Mohamed, 2013). As cited by Raatz & Bibus (2016), the American Heart Association recommends that eating fish at least twice a week must be part of a healthy diet.

Edible fishes are preserved through removal of moisture. According to Banglapedia (2014), fish drying is the removal of the water content of the fish, where the activity of the muscle enzyme is reduced to its minimum level. This method help in prolonging the shelf-life of the food product.

Fish salting and drying are one of the ancient and traditional methods that produce dietary food for human consumption. Air-drying and sun-drying are the most common methods in preserving fish due to its low cost and easy process.

As mentioned by the World Health Organization (2008), the predominant symptoms that may manifest upon consumption of food products contaminated by the fungal element or having mycotoxins are nausea, vomiting, diarrhea, thirst, and dilation of pupils.

Dried seafood is widely known as one of the basic commodities in the Philippines since it is available at a cheaper price for the economically challenged population of the society. It can also be kept for a long period of time because it underwent preservation. Thus, it is important for the consumers to know if there is a health risk from the consumption of dried fish and other seafoods.

METHODOLOGY

This chapter presents the information about the samples and methods used by the researchers to gain the needed data.

Research Design

To isolate and identify the fungi from dried fish and other seafoods sold in local market and selected supermarket in Batangas City, the researchers used a descriptive method in conducting the study. Washing method was used for the isolation of fungi. Portion of fungal growth were stained with Lactophenol Cotton Blue. Macroscopic

and microscopic observation were done to help identifying presence of fungi from the sample.

Research Locale

The samples were collected from old market and selected Supermarket (Citimart) in Batangas City. The Old Market is in one of the busy streets of Batangas City. It is near the roadways, where numerous vehicles pass by, making it unhygienic and disorganized. Citimart is located at the Baymall, Batangas City. The said place is air-conditioned, orderly arranged in sections (e.g. meat section, canned goods section, fruit section, and fish section), where the products are individually packed. The isolation of the fungi was conducted in the Instrumentation Room of Lyceum of the Philippines University- St. Cabrini School of Health Sciences Inc., located in Makiling, Calamba, Laguna.

Research Instruments

The materials used in the preparation of agar include: Sabouraud Dextrose Agar, distilled water, Erlenmeyer flask, analytical balance, stirring rod, spatula, and autoclave. For the isolation of fungi, materials used were distilled water, beakers, glass spreader, pipette, aspirator, and stirring rod. for the microscopic examination, Lactophenol cotton blue stain, glass slides, cover slip, inoculating needles, alcohol lamp, and microscope were used.

Data Gathering Procedure

Collection of Sample

10 g of each variety of dried seafood (pusit, tuyo, sapsap, dilis, daing) were obtained in the Local Market (Lumang Palengke) and Supermarket (Citimart) of Batangas City. The dried seafoods were sealed and labelled into polyethylene bags. Then, they were transported into the laboratory for isolation.

Preparation of Culture Media

A 65 g of Sabouraud Dextrose Agar was suspended in 1 liter of distilled or purified water. The suspension was heated with frequent

agitation and was boiled for one minute to completely dissolve the medium. It was sterilized by autoclaving at 121ºC for 15 minutes. The liquid suspension was cooled to 45º to 50ºC and was poured into sterile petri plate.

Isolation of Fungi

Washing method was done for the isolation of fungi. 10 grams of each dried seafoods (pusit, tuyo, sapsap, dilis, daing), obtained in the Old Market and selected supermarket in Batangas City, were transferred in 90 ml sterile distilled water through a beaker and stirred thoroughly for 5 minutes. 1 ml of the liquid suspension was plated on Sabouraud Dextrose Agar plates and spread evenly on the surface using a sterile glass spreader. The plates were incubated at room temperature for 7 days. Growths on the SDA plates were observed macroscopically by its colony morphology.

Microscopic Identification

Portion of fungal growth were transferred into a clean glass slide and 2-3 drops of Lactophenol Cotton Blue stain was added. A coverslip was carefully applied on the sample to avoid the formation of air bubbles. After 5 minutes of allowing the stain to penetrate through the transferred colony, it could stand on its own and was microscopically observed with low power objective and oil immersion objective for screening.

Data Analysis

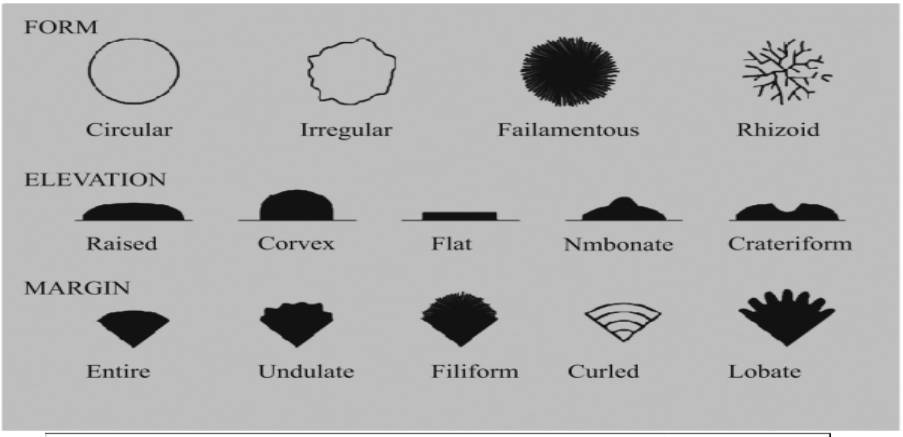
Upon conducting the experiment, the observed structure, forms, and morphological characteristics were gathered and described using standard charts to obtain the result.

Colony Morphology is a method that the researchers used to describe the characteristics of an individual colony of fungi growing on agar in a Petri dish. These characteristics can be used to help identify the fungi present in the sample. This includes the color, form, elevation, and margin.

Interpretations of the macroscopic appearance of the fungal growth were based on the standard chart (Figure 2) shown in Morphology and General Properties of Fungi (2016).

Spores were described based on the chart (Figure 2) obtained from the Morphology and General Properties of Fungi (2016).

Figure 1. Morphology and General Properties of Fungi.



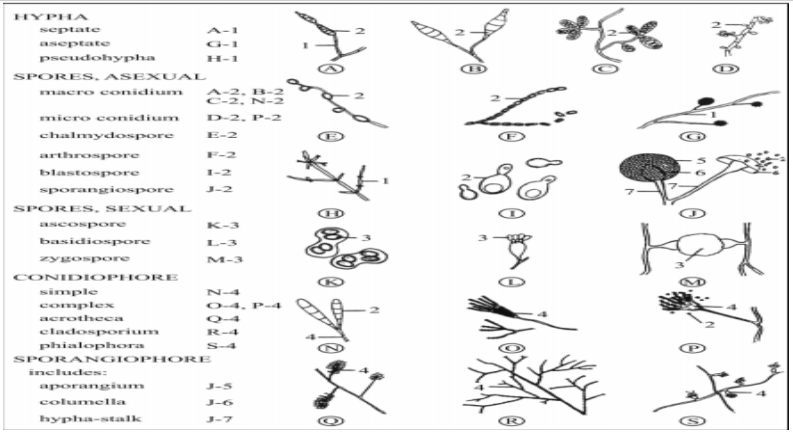


Figure 2. Standard Chart for Hyphae and Spores

RESULTS AND DISCUSSION

This chapter provides the results obtained from the experiment that was conducted, elaborated, and interpreted based on how the results were gathered.

Results

1. Morphological characteristics of the fungal growth

The color represents the pigmentation of the growth that had been isolated. The colors obtained were black, cream, white, and blue green. The form of an individual colony described the basic shape of the growth. The form obtained were irregular, rhizoid, filamentous, and circular. On the other hand, the elevation described the side view appearance of a colony, isolated fungi were convex, raised, pulvinate, and crateriform. The margins of obtained isolates were filiform, undulate, and filamentous, but the most common were undulate and filamentous. Margin or border describes the edge of the colony.

Figure 3. Presumptive *Aspergillus niger* on SDA

According to Jay, Martin & David (2005), one of the most common isolated fungi from food products is *Aspergillus*. *Aspergillus niger*. To identify this kind of fungus on media, there is the presence of yellow to white hyphae in colony, changing to black with development of conidia. Microscopically, *A. niger* can be detected by its septate hyphae. In addition, this fungus produces a very dark brown or a black spore, and has noticeable ridges (Metzger & Wilson, 2010).

From the experiment conducted, one of the presumptive fungal growth is *Aspergillus niger*. Figure 3 shows the appearance of the said fungi, producing darkly pigmented and has roughened spores (Tille, 2013). It is also stated that a yellow colony is formed that changed into black with dotted surface.

Figure 4. Presumptive yeasts on SDA

Presumptively, another fungal growth obtained was yeast that appears to be smooth and creamy, as seen in Figure 4. As cited by Vasanthakumari (2007), they appeared as round or oval. In culture media, creamy mucoid colonies are produced.

2. Isolation of fungal growth obtained from dried fish

Based on the results gathered, most of the isolates were molds. With the samples obtained from the local market, majority of the fungal growth were molds, for the samples from the selected supermarket molds also topped.

In comparison to the study of Logesh et al. (2012), the result of conducted Total Fungal Count showed a total of 6 fungi*, Penicillium sp.,*

Mucor sp., Rhizopus sp., and three *Aspergillus sp*., namely *A. niger, A.flavus,* and *A. fumigatus*. These findings were concluded due to a high level of moisture; thus, unsuitable for consuming. Moreover, Fredrick et al. (2015), reported twenty-three different fungal species in dried fish samples sold in Tuticorin, South East Coast of India dried fish markets. *Fusarium moniliformis, Absidia* sp*., Mucor* sp*., Penicillium* sp*., Aspergillus fumigatus, A. terreus, Aspergillus flavus, A. oryzae, Trichoderma* sp, *Geotricus candidum, A. sulphureus, A. terricola, A. awamori, A. flavipes, A. versicolor, A. tamari, Euroteum* sp, *Alternaria* sp, *A. parasiticus, A. sydowii* and *A. ochraceous* were the fungi associated. The said fungi, *Aspergillus, Absidia, Penicillium, Mucor*, and *Fusarium,* as well as *Chrysosporium, Cladosporium, Epidermophyton, Microsporum*, and *Trichophyton,* are classified as molds cited by McGinnis (2012).

3. Morphological characteristic of isolates using microscopic analysis

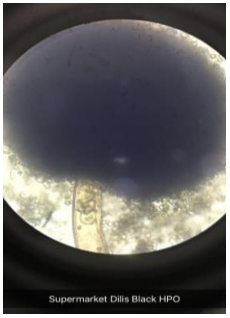


Figure 5. Presumptive *Aspergillus niger* under microscope

Presumptively, Figure 5 shows *Aspergillus niger*. Microscopically, the fungus showed septate hyphae, long conidiophores and smaller phialides, where long chains of brown to black, rough-walled conidia are produced.

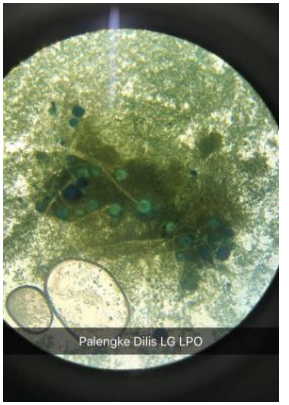


Figure 6. Presumptive *Aspergillus fumigatus* under microscope

Figure 6 shows the probable fungal growth, *Aspergillus fumigatus*. According to Tille (2013), it produces a fluffy to granular, white to blue-green colony. Microscopically, it is described by the appearance of long or short conidiophores, with a “foot cell” (T or L-shaped), and septate hyphae.

CONCLUSIONS

The researchers concluded that dried fish and other seafood sold in the Local Market and selected supermarket (Citimart) in Batangas City were contaminated with fungi. This implies that the environmental condition contributed much to the growth of microorganism in the dried fish. The most common morphological characteristic of the fungal colony obtained from the local market and supermarket were cream and white; in rhizoid and filamentous form; raised and pulvinate elevation; and undulate and filamentous margin, respectively. Individual colony of the isolated fungal growth appeared to be thread and cottony and was classified further into yeast and molds. The most common form was molds.

RECOMMENDATIONS

The knowledge in this study is just limited. It provides basic

information about the isolation and morphological identification of the presence of fungi based on a chart.

For further studies, the researchers recommend using other method of isolation of fungi, and different culture media. Utilize different temperature upon incubating the samples. Investigating on the pathogenesis of the isolated and identified fungi from the products may also be included. Discover more on the factors that can contribute for the growth of the microorganisms on the process of preservation to prevent contamination. Conducting quantitative approach will also be helpful for better understanding of the study.

For consumers, it is important to be meticulous when buying these products by carefully inspecting the dried fish to be bought. Note for the presence of white cottony growth on the dried fish or any discoloration which often signifies fungal contamination.

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