Identification of Microscopic Fungus in Gill of Skipjact Tuna 
(*Katsuwonus pelamis* L.) at Traditional Markets in Singaraja - Bali

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Abstract. Skipjack tuna is a medium-sized fish from family of *Scombridae* (tuna) which is currently widely sold in traditional markets in Singaraja - Bali. The freshness of the fish in the markets is strongly influenced by microorganisms especially microscopic fungus that contaninated from market environment. The purposes of this research were determined the number of microscopic polyculture colonies on skipjack tuna that sold in traditional markets in Singaraja - Bali, and microscopic fungus found in the gill of skipjack tuna (*Katsuwonus pelamis* L.). This research is used a qualitative approach in the form of descriptive explorative by isolating and identifying microscopic fungi. The subjects of this research were skipjack fish from traders in three traditional markets spread in Singaraja – Bali, such as Anyar Market, Banyuasri Market and Banyuning Market. While the object of this research was gill of skipjack tuna. As much as one gram of skipjack tuna was introduced into the sterile distillation as the first dilution and was carried out until 10⁻⁶ dilution to calculate the number of microscopic policulture colonies per dilution. Furthermore, microscopic fungus suspected to have different morphological characteristics were isolated to find pure isolates. Identification of each fungus as macroscopically and microscopically was performed after the purification. The results of this research indicated that the average of total microscopic polyculture colony in Anyar market amounts to 5.3 x 10⁵ cfu/ml; in the Banyuasri market of 2.2 x 10⁵ cfu/ml and Banyuning market at 6.4 x 10⁴ cfu / ml. There were 8 genera of microscopic fungus found in skipjack tuna in the three traditional markets in Singaraja city, namely Aspergillus, Penicillium, Rhizopus, Mucor, Trichoderma, Culvularia, Verticillium and Cladosporium. All the fungus that found as contaminant from the markets environment.

1. Introduction

Singaraja is one of the town that located in the northern part of Bali island. This town is very close to the beach. Singaraja’s people are very interesting to eat food that comes from the sea, one of them is fish. It selected by people in singaraja because it contains high enough nutritional value. In some markets in singaraja city, the most popular fish for consumption is skipjack. One type of fish that is a marine biological resource in Bali is skipjack tuna that has a high economic value, both for local consumption and for export.

Based on the data of [5], The fish consumption for Bali province in 2010 to 2014 has increased 8.21%. The high interest of skipjack consumptions in Bali especially in Singaraja make the sellers in the market are doing various ways to extend the shelf life of the fish, ranging from preservation and so forth. But sometimes, bad handling and the condition of the markets cause skipjack's tuna
(Katsuwonus pelamis L.) decay very quickly. This is supported by the geographical location of Indonesia which includes tropical areas and has a high enough air humidity, making it suitable for the growth of microbes for food destruction. Process of decay in fish is generally caused by enzyme activity, chemical activity and microorganisms [7].

Body of the skipjack that susceptible to microorganisms is the gill. This section is a respiratory organ that binds oxygen and releases respirable carbon dioxide. In the gills of the skipjack is also have direct contact with the pollutants and the first organ directly related to the food consumed skipjack enters into the body, so this gill part becomes the main target of the growth of rotting microorganisms.

Microorganism is all small, invisible living things. One of the microorganisms such as microscopic fungus can be the cause of decay in skipjack. Most microscopic fungi are able to produce mycotoxins which are secondary metabolites that can damage the body of the fish so it becomes rotten (Sarjito, 2013). Based on the problems that have been described that the decay can occurs in skipjack is caused by microorganisms especially microscopic fungus from skipjack (Katsuwonus pelamis L.) on traditional markets in Singaraja, Bali.

2. Research Method

2.1 Tools and materials
Equipment used in this research is surgical board, autoclave, pipette, tweezers, incubator, analytical scales, glass object, needle ose, microscope, refrigerator, scissors, bunsen lamp, petri dish, test tube, measuring cup, laminar air flow and plastic bags, while the materials used in this research were gills of skipjack (Katsuwonus pelamis L.), Dextrose Agar (PDA), among others, potatoes, glucose (dextrose) and colorless agar, Media Malt Extra Agar (MEA), emersi oil, sterile distilled water, 70% and 96% alcohol.

2.2 Preparation of Skipjack Tuna Samples
Skipjack fish with an average body weight of 150-200 grams of which 9 samples were taken, sampling was conducted at three different locations representing the traditional market location in Singaraja town, Bali such as Anyar Market, Banyuasri Market and Banyuning Markets. Each of the market was taken for two fishes. The skipjack samples are taken randomly, and the test organ is gill. The sample was directly taken to Microbiology Laboratory, Department of Biology, F.MIPA, Ganesha University of Education (Undiksha) for examination of microscopic fungus to identification.

2.3 Isolation of Microscopic Fungus from Tuna Fish Samples
Isolation of fungi begins by taking gill samples and dissolved in sterile water as much as 90 ml to obtain a dilution rate of 10 times (10⁻¹). The sample was diluted further until the highest dilution rate was obtained by 10⁻⁶. A 1 ml sample with a dilution rate of 10⁻¹ to 10⁻⁶. After that it dispersed on the surface of the PDA medium, incubated at room temperature for 2-5 days. Then in the fifth day, count the colony and the suspected microscopic fungus colony was purified again on PDA and MEA media. Identification is done by looking at the macroscopic and microscopic forms of the Microscopic fungus and matched it by references [1].

2.4 Data Analysis
Data analysis of this research was done with qualitative approach. data on the number of microscopic fungus colonies in each dilution is made in tabular form, then the results were analyzed descriptively. the characteristics of each microscopic fungi isolate were also submitted in table form and described

3. Result and Discussion
The number of microscopic fungi colonies from skipjack (Katsuwonus pelamis L.) at traditional market in Singaraja, presented in table 1. form as follows.
Table 1. The Number of Microscopic Fungus Colonies from Gill of Cakalang (*Katsuwonus pelamis* L.) at Traditional Market in Singaraja Town, Bali

| Sample          | Anyar Market (AM) | Banyuasri Market (BM) | Banyuning Market (ByM) |
|-----------------|-------------------|-----------------------|------------------------|
| Skipjack 1      | 3.9 x 10^5        | 11 x 10^3             | 59 x 10^1              |
| Skipjack 2      | 1.5 x 10^4        | 7.0 x 10^3            | 2.0 x 10^3             |
| Skipjack 3      | 1.4 x 10^5        | 5.1 x 10^3            | 3.2 x 10^3             |
| **Total Number of Colonies** | **5.3 x 10^5** | **2.2 x 10^5** | **6.4 x 10^4** |

Based on table 1, it can be seen that the number of microscopic fungi colonies isolated from the skipjack tuna (*Katsuwonus pelamis* L.) per ml at traditional market in Singaraja Town. The highest number of microscopic fungi colonies in PA1 were 3.9 x 10^5 CFU / ml and the lowest number of microscopic fungi colonies were in PBy3 of 3.2 x 10^3 CFU / ml. The total number of microscopic fungi colonies from the three skipjacks (*Katsuwonus pelamis* L.) at Anyar Market is 5.3 x 10^5 CFU / ml, at Banyuasri Market 2.2 x 10^5 CFU / ml and at Banyuning Market 6.4 x 10^4 CFU / ml.

Table 2. The Genus of Microscopic Fungus Isolated from the Gills of Skipjack (*Katsuwonus pelamis* L.) at Traditional Market in Singaraja Town.

| Market        | Isolate | Result of Genus Identification |
|---------------|---------|--------------------------------|
| 1 (Anyar Market) | PA1     | *Aspergillus*                  |
|               | PA2     | *Penicillium*                  |
|               | PA3     | *Penicillium*                  |
|               | PA4     | *Rhizopus*                     |
|               | PA5     | *Mucor*                        |
|               | PA6     | *Trichoderma*                  |
|               | PA7     | *Aspergillus*                  |
|               | PA8     | *Aspergillus*                  |
| 2 (Banyuasri Market) | PBs1   | *Penicillium*                  |
|               | PBs2   | *Curvularia*                   |
|               | PBs3   | *Verticillium*                 |
|               | PBs4   | *Cladosporium*                 |
| 3 (Banyuning Market) | PBy1   | *Aspergillus*                  |
|               | PBy2   | *Cladosporium*                 |
|               | PBy3   | *Penicillium*                  |

The most common microscopic fungus genus is found in skipjack tuna in the three traditional markets namely the genus *Aspergillus* and *Penicillium*. The genus of *Aspergillus* can be found in Anyar Market and Banyuning Market, while Banyuasri Market is not found. The *Penicillium* genus can be found in all three markets. The genus *Cladosporium* can be found in the Banyuasri and Banyuning markets, whereas the Anyar Market is not found in this genus. The genera *Rhizopus*, *Mucor* and *Trichoderma* are found only in the Anyar Market, whereas in Banyuasri and Banyuning markets are not found these three genera. The genus *Curvularia* and *Verticillium* are not found in Anyar Market and Banyuning Market, but this genus is found in Pasar Banyuasri.
Table 3. Physical Parameters in Traditional Market in Singaraja Town

| Market    | Air Humidity (%) | Light Intensity (Lux) | Temperature (°C) |
|-----------|------------------|-----------------------|------------------|
| Anyar     | 71               | 240                   | 25.4             |
| Banyuasri | 80               | 200                   | 28.5             |
| Banyuning | 72               | 250                   | 26.2             |

Based on table 3, it can be seen that each market where sampling has air humidity, light intensity and temperature are different, but the difference of this parameters were not significant. This condition could affect the condition of the skipjack samples, because the microbe that grew on the skipjack depends on the physical condition of each market and the condition of the skipjack itself.

Based on the research that has been done, it is known that the skipjack fish on Anyar Market has the highest number of microscopic fungus colony of 5.3 x 10^5 CFU / ml. This happens because it is influenced by several factors, including the state of the skipjack itself and the physical condition of the market when sampling is taken. According to the observations that have been made when sampling the skipjack fish, the atmosphere of Pasar Anyar is filled with many people who were buying and selling transactions and the location of selling fish traders close to landfills.

At Anyar Market, it was found that air humidity at the time of sampling that is 71% with light intensity 240 Lux and temperature at that time that is 25.4°C. Such conditions may spur the growth of microscopic fungi, because low-grade fungus could grow optimally at room temperature and high humidity. It was in line with that proposed by [4], where microscopic fungus generally required an environment with 70% - 90% moisture to develop and optimum fungus temperature to be able to grow generally between 22°C - 30°C.

The second sampling location was at Pasar Banyuasri. The total number of polyculture microscopic fungus colonies in Banyuasri Market was less than in Anyar Market which is 2.2 x 10^5 CFU/ml. This occurred because when sampling, the condition of the skipjack and market conditions were in normal condition. Based on the observations that have been made, that people who made buying and selling transactions at that time was not much. Handling sellers against skipjack was good. Skipjack is placed in a large box containing ice and closed, then the chances of microscopic fungus contamination from the environment against the skipjack tuna. So that, the number of polyculture of fungi and the number of pure isolates in skipjack fish was less than Anyar Market. It was in accordance with the proposed [7]. The amount of growth of a microbe was strongly influenced by human roles and activities. Through good and correct handling, it can suppress the population of harmful microbial growth. Handling is considered good by cold chain implementation, keeping fish at low temperatures, for example by using this ice is done to reduce the growth of microorganisms that can slow the process of decay in fish.

The third Sampling of skipjack (Katsuwonus pelamis L.) was in Banyuning Market. The total number of microscopic fungus colonies on the Banyuning market was at least in comparison with Anyar Market and Banyuasri Market at 6.4 x 10^4 CFU/ml. According to observations that have been done, this happens because when sampling, the condition of the skipjack and the condition of the market environment were in normal situation. Because at that time the market situation and skipjack buyers are not crowded. The fish sellers in Banyuning Market have done a good handling of skipjack.

Skipjack (Katsuwonus pelamis L.) at Anyar Market is indicated to be faster decayed by fungus, because the number of microscopic fungus colonies exceeds the maximum limit of microbial contamination in fresh fish that is 5 x 10^5 CFU / ml [11]. Meanwhile, skipjack fish at Pasar Banyuasri and Pasar Banyuning, the total number of microscopic fungus colonies were still below the maximum limit set by the Indonesian National Standard Board. So that the skipjack fish in these two markets have better quality than fish in Anyar Market.
The skipjack fishers mostly put the fish on the tray with the condition of the fish is not closed, so the fish are exposed to air that has been contaminated with microbes and cause the growth of microbes in large quantities and accelerate the occurrence of decay in fish, this is in accordance with the proposed [12], where the growth of microbial quantities and the process of fish quality decline is accelerated by poor handling or arrest, inadequate facilities and sanitation and limited distribution and marketing facilities.

The microscopic fungus that dominates growth in skipjack (Katsuwonus pelamis L.) on traditional markets in Singaraja City was the genus of Aspergillus and Penicillium. The genus Aspergillus is found in skipjack fish from Pasar Anyar and Pasar Banyuasri. According to [6], the genus Aspergillus belongs to the phylum of Ascomycota, the order of Eurotiales, and the Trichocomaceae family. The genus Aspergillus generally has white, yellow or green base feathers with a thick brown to blackish conidiospora layer. This genus is found in nature as a saprophyte, grown in a tropical region with high moisture, its original habitat in the soil. The genus Aspergillus can also be found in cereals (maize, sorghum, rice, wheat) and various stored food items. Because in this market, fish’s sellers located near from vegetable’s sellers.

In this research also identified genus of Curvularia, Verticillium and Cladosporium fungi. These three genera are found in the Banyuasri Market and the Cladosporium genus is also found in Banyuning Market. The original habitat of these three fungi is generally on soil and grass. The Cladosporium genus can also be found in foodstuffs.

Based on the exposure, it can be seen that of the 8 genera identified, the genus Aspergillus, Penicillium, Rhizopus and Mucor were fungal pathogen causes decay in skipjack (Katsuwonus pelamis L.) and mostly found in land. These four genera of fungi are capable of producing mycotoxins that cause rotting in skipjack. The genus Aspergillus capable of producing mycotoxins in the form of aflatoxins that attack the fish can cause fish to suffer physical damage until decay occurs. According Setiarto [11], Penicillium genus could produce mycotoxins in the form of okratoksin that can damage the fish body, causing decay in fish. According to [9] the genus Rhizopus and Mucor could cause zygomycosis and mucormycosis in fish, which can cause decay in fish because of physical, chemical, odor, color and texture damage to the fish. The genus of Curvularia, Verticillium and Cladosporium fungi did not produce mycotoxin compounds that endanger the skipjack, so this fungus plays a small role in the decay of fish [11].

4. Conclusion
Based on the results of the research, data analysis and discussion has been presented, it can be drawn conclusion as follows.
1) The total number of microscopic fungi colonies isolated from the skipjack tuna (Katsuwonus pelamis L.) on the Anyar Market market of 5.3 x 10^5 CFU / ml, at Banyuasri Market of 2.2 x 10^5 CFU / ml and on Banyuning Market 6.4 x 10^4 CFU / ml.
2) The total number of genus of microscopic mushroom isolated from gill of skipjack (Katsuwonus pelamis L.) is 8 genus of fungi. The eight genera of the fungus are, the genus Aspergillus, Penicillium, Rhizopus, Mucor, Trichoderma, Curvularia, Verticillium and Cladosporium. All the fungus that found as contaminant from the markets environment

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