

The Isolation of *Staphylococcus epidermidis* Bacteria in White Snapper Salted Fish (*Lates calcalifer*) from Sibolga City, North Sumatera Province

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Abstract

Salted fish is vulnerable to contamination by microbial. *Staphylococcus epidermidis* is one of the bacteria that can contaminate the salted fish. This research aims to isolate the *Staphylococcus epidermidis* bacteria contamination in salted white snapper fish sold in Sibolga City, North Sumatera Province. The sample used was white snapper salted fish, amounting to 10 samples from 10 traders. The isolation of *Staphylococcus epidermidis* was carried out using the Carter method. White snapper salted fish are mashed using a blender, then planted on *Nutrient Broth* (NB) as a bacterial growth media. Furthermore, identification of bacterial colonies grew using gram staining, *Manitol Salt Agar* (MSA) media, *Blood Agar Plate* (BAP) media, catalase test and confectionery media (Manitol and Glucose). The data obtained were analyzed descriptively. The results of this study suggest that *Staphylococcus epidermidis* contains bacterial contamination. Based on the data collected, it can be concluded that 7 out of 10 samples of white snapper salted fish sold in Sibolga City, North Sumatera Province, are contaminated with 70 percent *Staphylococcus epidermidis* bacteria.

Keywords: salted fish; *Staphylococcus epidermidis*; contamination

Introduction

Sibolga is one of the cities in the province of North Sumatera, recognized as the most abundant fish producing area in the world, where fisheries production in Sibolga has increased annually (Geffken *et al.*, 2017). Sibolga is a town on the west coast of Sumatera with such great potential in the fisheries sector and it can also be said that the majority of the Sibolga communities' economy is driven by marine fishing (Sihite, 2013). Fish is a significant source of food for the body. Fish is also a food product that experiences decay and damage very quickly (Kamal *et al.*, 2016). Traditionally, society often does the protection of fish by salting (Agustina *et al.* 2013). Fish preservation by salting is a way for fish not to decay quickly (Salosa, 2013). Salted fish products are also popular among the people, and

salted fish is consumed in the community because it is easy to get, and also the price is relatively cheap so that people can enjoy it. Drying salted fish aims to increase the durability of the fish (Wardani & Mulasari, 2016).

Viewed from the processing, salted fish is typically processed using traditional methods in Indonesia (Purnomo *et al.*, 2017). The method of salted fish processing that is usually carried out by fishermen until now has not been hygienically carried out. It is very susceptible to the pollution that affects the shelf-life of fish when drying under direct sunlight (Marpaung, 2015). Viewed from the low level of fish freshness and unsafe food health and its processing techniques, the implementation of sanitation and hygiene in conventional fishery products processing technology is graded as poor

(Akerina, 2018). The traditional way of preserving fish is aimed at reducing the amount of water content found in the fish's body, so this approach does not provide an opportunity for bacteria to multiply. In order to obtain preserves of high quality, good care during the preservation process is required, such as: maintaining the cleanliness of the tools and materials used, using fresh fish and using clean salt (Tuyu *et al.*, 2014).

Most of the causes of infections caused by *Staphylococcus epidermidis* and *Staphylococcus aureus* have gained public attention because there has been a rise in infections caused by this type of bacteria worldwide in several years. *Staphylococcus epidermidis* is one of the bacteria that causes disease in humans, which is usually found in processed fishery products (Chessa *et al.*, 2015). One of the signs of opportunistic pathogens caused by *Staphylococcus epidermidis* bacteria is the incidence of infection in humans who have weak immunity (Karimeila *et al.*, 2018). *Staphylococcus* food poisoning is usually characterized by the sudden onset of symptoms including nausea, vomiting, cramps of the stomach and diarrhea (Asao *et al.*, 2003). According to Faridz *et al.* (2007) things like this need to be considered mainly from the microbiological quality of food. The product is the number and type of microorganisms found in these foods. Contamination caused by *Staphylococcus epidermidis* bacteria in salted fish must be further tested in the Laboratory before consumption by the public. However, isolation of *Staphylococcus epidermidis* has never been done in white snapper salted fish in Sibolga City. Based on these problems researchers are interested in isolating *Staphylococcus epidermidis* bacteria in white snapper salted fish.

Materials and Methods

The study was conducted in November 2019 at the Microbiology Laboratory at the Faculty of Veterinary Medicine, Syiah Kuala University. Sample fish were collected from the salted fish market in Sibolga City, Province of North Sumatra. Ten samples of salted white snapper (*Lates calcalifer*) were used that was gathered from Sibolga City salt fish traders.

Taking into account the venue, the sampling method is completed. The location taken is a highway that often passes through vehicles such as motorcycles and cars, making it easy for customers to access. This study uses samples of white snapper salted fish (*Lates calcalifer*) for a total of 10 fish from each trader in the town of Sibolga. Every fish sample taken was put in one plastic box and another. The salted fish were then brought to the Faculty of Veterinary Medicine's Microbiology Laboratory at Syiah Kuala University.

Research procedures

The procedure of isolating *Staphylococcus epidermidis* was done by using Carter's method (1987). White snapper salted fish are mashed using a blender, then planted on the media *Brut Nutrient* (NB) as a medium for bacterial growth. Furthermore, identification of bacterial colonies that grew using gram staining, *Manitol Salt Agar* (MSA) media, *Blood Agar Plate* (BAP) media, catalase test and confectionery media (Manitol and Glucose).

Bacteria isolation

Fish samples were weighed as much as 2 grams, taken from 0.5 grams of the fish's head, 0.5 grams of the tail, 0.5 grams of the fish's right body, and 0.5 grams of the fish's left body. Besides, they were blended with a blender for 30 seconds until the texture of the fish meat is smooth, a sterile swab inserted into the blender. Next, enter the sterile swab into the media *Nutrient Broth* (NB). The tubes containing 10 ml *Nutrient Broth* (NB) media were homogenized and incubated at 37°C for 24 hours, then the bacterial suspension was taken from the *Nutrient Broth* (NB) media, then scratched on *Manitol Salt Agar* (MSA) media. Bacteria that were inoculated into the *Blood Agar Plate* (BAP) were derived from *Manitol Salt Agar* (MSA) media with the T scratch technique and incubated at 37°C for 24 hours. *Staphylococcus epidermidis* positive samples were planted on slanted *Nutrient Agar* (NA) media.

Gram staining

The process done in Gram staining is the disinfection of glass items with 70 percent alcohol. Physiological NaCl is dripped onto glass surfaces during disinfection. The ose loop is heated by

including it on a methylated lamp. The bacterial colony is seized with ose on the Blood Agar Plate media, then positioned and homogenized on a glass object given physiological NaCl by forming a 1 cm diameter circle. Then the colony is left aerated to dry. The preparation is set by putting the glass item 2-5 times on the spirits lamp. The set preparations are then given 1-2 minutes of violet crystal dyeing.

Furthermore, the preparation is washed with running water. The preparations were then flooded with Lugol for 1 minute. After that, the preparation is washed with 96% alcohol for 5-10 seconds. Next, The preparations were saturated with safranin and left for 1 minute, then washed with running water and aerated. After drying, the preparations are dripped with emersion oil and microscopically examined at a magnification of 10 times.

Catalase test

Catalase testing was performed by taking separate bacterial colonies from the *Manitol Salt Agar* (MSA) and *Blood Agar Plate* (BAP) media, then exposing the bacterial colonies to glass objects, dropping H₂O₂ 3% in glass objects on bacterial colonies. The formation of air bubbles shows positive results which indicate the bacteria produce the enzyme catalase. If there are no visible air bubbles, the outcome is negative.

Hemolysis test

Test of hemolysis was carried out on medium *Blood Agar Plate* (BAP) with the aid of Ose, then inoculated on *Blood-Agar Plate* (BAP) mediums using the medium *Manitol Salt Agar* (MSA) and T streak technique. Media *Blood Agar Plate* (BAP) was incubated in an incubator for 24 hours at 37°C.

Sugar test (mannitol and glucose)

A separate bacterial colony of the manitol is taken by sterile ose and inserted in a mannitol-bearing testing tube by extracting sterile ose homogenously until the bacterial colony in the Ose became homogenous in the mannitol environment. It is taken into a separate bacterial colony by using sterile Ose. Next Bacteria Colony Incubate 24 hour mannitol at 37°C. The same is correct for glucose.

Data analysis

The results of colony growth from the isolation of the *Staphylococcus epidermidis* bacteria were analyzed descriptively.

Results

Salted fish used in this analysis stood at ten samples from 10 traders around Sibolga Town. Table 1 shows the results of analyses of *Staphylococcus epidermidis* contamination in white snapper salted fish from 10 samples incubated at 37°C for 24 hours, can be seen in Table 1.

Data in the Table 1 consist of 10 salted fish samples obtained in Sibolga City showed a high prevalence rate of *Staphylococcus epidermidis* contamination rate which reached 70% where seven positive samples were contaminated with *Staphylococcus epidermidis* ie samples at traders 1, 2, 5, 6, 7, 8, and 10 while samples 3, 4, and 9 showed positive results that there were other bacterial contamination, besides *Staphylococcus epidermidis* other bacteria were also able to live on salted fish.

Table 1. Observation Result of *Staphylococcus epidermidis* contamination on the samples

No	Sample	Bacteri
1.	P 1	<i>Staphylococcus epidermidis</i>
2.	P 2	<i>Staphylococcus epidermidis</i>
3.	P 3	non <i>Staphylococcus epidermidis</i>
4.	P 4	non <i>Staphylococcus epidermidis</i>
5.	P 5	<i>Staphylococcus epidermidis</i>
6.	P 6	<i>Staphylococcus epidermidis</i>
7.	P 7	<i>Staphylococcus epidermidis</i>
8.	P 8	<i>Staphylococcus epidermidis</i>
9.	P 9	non <i>Staphylococcus epidermidis</i>
10.	P 10	<i>Staphylococcus epidermidis</i>

Discussion

The number of bacteria in fish is also highly influential on the freshness level. Damage to fish and processed goods such as salted food caused by spoilage bacteria has symptoms of damage such as a pungent smell, fly-infested, and unpleasant taste changes. The pungent odor is caused by the release of certain chemicals produced by spoilage bacteria such as NH_3 and cadaverine. Stages of damage occur because the protein produces NH_3 and H_2S with the help of peptides, free amino acids and vitamins, causing a specific odor discharge (Fifendy *et al.*, 2017). Damage to processed fish products can be seen from the formation of brownish red or yellow spots on the surface of the fish's body and the presence of mucus (Marpaung, 2015). The bacteria that can survive in an atmosphere that has a high salt content are called *halophilic* bacteria, a group of bacteria that are included in *halophilic* bacteria (Fifendy *et al.*, 2017).

Also, it can be considered both from the way of handling, sanitation, biological factors, environmental temperature, fish transportation equipment, and storage space can also affect the quality of salted fish produced (Riski *et al.*, 2017). Based on observations made in the field, fish that are ready for sale are hanged at salted fish traders for > 12 hours. Moreover, the location of the salted fish sales location is right on the side of the road, so that bacterial contamination can occur through the air that is around the merchant's place. The level of bacterial contamination illustrates how the condition of sanitation because the purpose of sanitation is to prevent the entry of contaminants into food and processing equipment used in food processing, and prevent recontamination (Susianawati, 2007), so that prevention of contamination can be done if sanitation from individuals can be improved.

Prevention of *Staphylococcus epidermidis* bacterial contamination can be done by paying more attention to the processing methods of processed salted fish products that are good starting from the process of selecting fresh fish, selecting clean salt, and weeding, cleaning and drying the fish done by paying attention to its cleanliness. Storage that is cool, dry, and has proper air ventilation is a desirable condition for storing salted fish products. Of course, salted fish when marketed must use a closed package

to reduce the danger of contamination and pay attention to the level of maturity of the fish when cooking. In the process of salted fish, processing starts from weeding, washing, salting and drying stages. Where in the salting stage, it can be noted from the amount or content of salt used, the length of the salting period, as well as the drying time, are factors that can affect the final results of salted fish to be produced can be seen from the drying process. The selection of salt used as one of the preservation stages is also a determinant of the success of the processed fish salted result, which is salt with a level of smoothness, purity and right salt concentration and seen from the fish material used wherein getting good quality is determined by the freshness of the fish, the content and thickness of the fish (Marpaung, 2015).

Preserving fish by combining the stages of salting and drying can provide a double benefit where salted fish products will be more durable because the purpose of the fish salting and drying stages is to reduce the level of water contained in the body of the fish. The close relationship between low water content and the durability of processed fish products will result in better durability of processed fish products. This is because, in the occurrence of enzymatic and chemical processes as well as the growth of bacteria, a certain amount of water is needed. Decreased water content in the material will inhibit the growth of spoilage bacteria.

Based on the results of bacterial isolation on the media of *Manitol Salt Agar* (MSA) which was planted at 37°C for 24 hours, it was marked by a change in the color of the media from red to beige and visible convex-shaped colonies with flat edges. Jamaluddin *et al.* (2016) which states to be able to distinguish between *Staphylococcus epidermidis* and *Staphylococcus aureus*, can use the media of *Manitol Salt Agar* (MSA). Where the *S. aureus* bacteria can ferment Manitol so that it can change the *Manitol Salt Agar* (MSA) medium from red to golden yellow while the *Staphylococcus epidermidis* cannot ferment Manitol, shown in Figure 1.

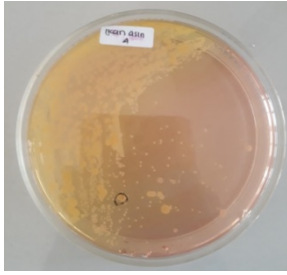


Figure 1. *Staphylococcus epidermidis* colony from Trader 1 sample on *Manitol Salt Agar* (MSA) media.

Staphylococcus sp. Bacteria are facultative anaerobic bacteria and do not form spores. Growth temperature ranges from 6.5°C-40°C with optimal growth occurring at 37°C. This bacterium can grow in the range of pH values 4.2 - 9.3. *Staphylococcus* sp is a pathogenic bacterium that has the ability to produce enterotoxins which can be a disease caused by consuming contaminated food (Pailu and Budiarmo, 2017). Enterotoxins are enzymes that can survive in hot conditions and are resistant in an alkaline atmosphere in the intestine which can cause food poisoning (Rahayu *et al.*, 2014). Prevention of bacterial contamination of *Staphylococcus* sp can be done by observing the storage temperature of fish and when the fish is cooked until cooked, usually poisoning when consuming food can occur because the food is not stored at high enough temperatures ($\geq 60^\circ\text{C}$) or cold enough ($\leq 7.2^\circ\text{C}$) (Hayu, 2018).

Salted fish sold in Sibolga City should be paid more attention both in terms of the place of sale and during processing so that contamination can be reduced. Bacterial growth on *Manitol Salt Agar* (MSA) media which was then identified by Gram staining. From the gram staining results obtained, bacterial colonies look purple, which states the bacteria are gram-positive. Gram-positive bacteria have a thicker peptidoglycan wall compared to gram-negative bacteria so that at the time of staining, gram-positive bacteria can bind to a violet crystal dye. Gram staining results observed under a microscope show the form of bacteria microscopically is a way to see the characteristics of a round bacterium. In this research, it was found that the bacteria were round, which meant that they belonged to the cocci group (Safrida *et al.*, 2012) as shown in Figure 2.

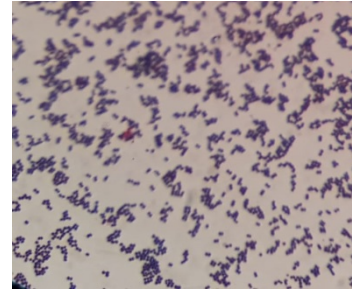


Figure 2. The results of Gram *Staphylococcus epidermidis* staining

Salted fish samples that have been able to grow on the media of *Manitol Salt Agar* (MSA) are then further identified by the catalase test, then to see the ability of bacteria in blood hemolysis is done by using the media *Blood Agar Plate* (BAP) followed by planting on a sugar medium (*Mannitol and Glucose*). Based on the results of the examination on the catalase test, hemolysis test and sugar test there are seven samples of traders showing positive results of the *Staphylococcus epidermidis* bacteria, that is, traders samples 1, 2, 5, 6, 7, 8, and 10. The positive catalase test results in the presence of air bubbles. The result of the hemolysis test is the formation of the γ -hemolysin zone and the sugar test shows negative mannitol and positive glucose.

Conclusion

Tests from 10 salted fish traders in Sibolga City showed that 7 out of 10 salted fish samples tested 70 percent contaminated with *Staphylococcus epidermidis* bacteria in white snapper (*Lates calcalifer*).

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Author Contribution and Competing Interest

Rafika Lestari and MD carried out the research. DD and RR conceptualized this research. NN, AH, and GR revised the manuscript. All authors read and approved the final manuscript. There was no competing interest related to the conduct of this study.

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