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ISOLATION OF PROTEOLYTIC AND LIPOLYTIC BACTERIAS IN PRESERVED *Rastrelliger canagurta* WITH CABBAGE LETTUCE (*Lactucca sativa*) ENSILING FERMENTATION AND KEPAYANG SEED (*Pangium edule Reinw*)

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Abstract. *Rastrelliger canagurta* are one kind of fish that easily get damage because of their spacious body tissue, so that the microbe can use the fish flesh for their substrat. Therefore it is need a special handling in order to prevent damaged and putrided. One of the biologycal preservation method for fish is ensiling fermentation preservation, it is a preservation that use vegetable, i.e. : cabbage lettuce (*Lactucca sativa*) leaf dan kepayang seed (*Pangium edule Reinw*). Proteolitic and lipolytic bacterias that found in fish body have a potention to putrid the fish flesh. The purpose of this research are: 1) to isolate the bacterias in preserved *Rastrelliger canagurta*; 2) to know the proteolytic bacterias in preserved *Rastrelliger canagurta*; 3) to know the lipolytic bacterias in preserved *Rastrelliger canagurta. Rastrelliger canagurta* samples take from markets in Malang city. Fish samples preserved by combine cabbage lettuce (*Lactucca sativa*) ensiling fermentation dan kepayang seed (*Pangium edule Reinw*) then saved for 21 days. 25 grams *Rastrelliger canagurta* sample diluted in 225 ml peptone broth 0.1% solution to get 10^{-1} dilution factor of suspension. Then the suspension diluted gradually to get 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilution factor of suspension. Each suspension inoculated 0.1 ml on Plate Count Agar (PCA) medium and incubated in 37^0C during 1-2x24 hours. Every bacteria species that grow on PCA medium were isolated, then proteolytic and lipolytic characters of each bacteria were confirmed. Protein hydrolysis index and lipid hydrolysis index were counted. The research result shows: 1) there are 9 isolate of bacterias found in the fish samples; 2) there are two isolate of proteolytic bacteria in preserved *Rastrelliger canagurta*; 3) there are nine isolate of lipolytic bacteria in preserved *Rastrelliger canagurta.*

***Keywords**: Rastrelliger canagurta, cabbage lettuce leaf, kepayang seed, proteolytic bacteria, lipolytic bacteria.*

1 Introduction

Fish and waters product are one most alternative food in the future that can be consumed by society, because this commodity easier to get in Indonesian marine. The fish can easily get damage and abstruct the fish market, this is usually make great loss

specially when harvest fish product. Because of that, therefore needed processing to keep the product in good condition, nutrient, taste, smell, shape and well preserved (Adawyah, 2007).

Rastrelliger canagurta are one kind of fish that easily get damage because of their spacious body tissue, high water contens and a lot of nutrient, so that the microbe can use the fish flesh for their substrat. Therefore it is need a special handling for *Rastrelliger canagurta* in order to prevent damaged and putrided.

There are so many way to do food preservation method for meat and the other product in order to prevent putrided by microbe. Among those preservation method, biological preservation method is the most suggestion to do, because beside it used original preservation substance, this method is more easier and cheaper so this method can be done by common people. One of the biologycal preservation method for fish is ensiling fermentation preservation, it is a preservation that use vegetable, i.e. : cabbage lettuce (*Lactucca sativa*) leaf dan kepayang seed (*Pangium edule Reinw*).

When ensiling fermentation preservation occur, there are a lot of proteolitic and lipolytic bacterias that found in fish body have a potention to putrid the fish flesh. Therefore it is needed to isolate proteolytic and lipolytic bacterias in preserved *Rastrelliger canagurta* (*Rastrelliger canagurta*) with cabbage lettuce (*Lactucca sativa*) ensiling fermentation and kepayang seed (*Pangium edule Reinw*) and to confirm the protein and lipid hydrolysis index.

The purpose of this research are: 1) to isolate the bacterias in preserved *Rastrelliger canagurta*; 2) to know the proteolytic bacterias in preserved *Rastrelliger canagurta*; 3) to know the lipolytic bacterias in preserved *Rastrelliger canagurta*.

2 Research Method

2.1 Research Tools

The tools used to prepare materials research is analytical scales, spoons, plastic containers, knives, glass jars, bean-breaking tool, pick a tool, box stereofoam. The tools used for the isolation of lipid and protein hydrolysis bacteria is a blender, test tubes, Erlenmeyer, petridish, incubators, mortar-pistil, colony counters, long slide.

2.2 Materials Research

The basic ingredients used in this study is *Rastrelliger canagurta* (Rastrelliger canagurta) with an average weight of 100 grams, cabbage lettuce (*Lactucca sativa*) and kepayang seed (*Pangium edule Reinw*). Chemicals used for the isolation of lipid and protein hydrolysis bacteria are Plate Count Agar (PCA) medium, peptone broth 0,1 %, Nuterien Agar + Lipid (NAL) medium and Skim Milk Agar (SMA) medium.

2.3 Research Methods

The method is performed in this study is exploratory deskripstif method. *Rastrelliger canagurta* (*Rastrelliger canagurta*) samples preserved by combine cabbage lettuce (*Lactucca sativa*) ensiling fermentation dan kepayang seed (*Pangium edule Reinw*) then saved for 21 days. 25 grams *Rastrelliger canagurta* sample diluted in 225 ml peptone broth 0.1% solution to get 10^{-1} dilution factor of suspension. Then the suspension diluted gradually to get 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilution factor of suspension. Each suspension inoculated 0.1 ml on Plate Count Agar (PCA) medium and incubated in 37^0C

during 1-2 x 24 hours. Every bacteria species that grow on PCA medium were isolated on SMA medium to confirm the protein hydrolysis index and NAL medium to to confirm the lipid hydrolysis index.

3 Results and Discussion

This research result show the bacterias that found in *Rastrelliger canagurta* that had been preserved are nine species bacteria which are unknown whether it's lipolytic or proteolytic bacteria, namely A, B, C, D, F, G, H and I. The dominant isolated bacteria grown in PCA medium is isolate bacteria B, F, and I. Further testing is testing the ability of hydrolyzing protein and lipid on all types of bacteria isolates were found to determine the species of bacteria which are proteolytic and lipolytic.

After testing the ability of lipid hydrolysis by inoculated each isolates of bacterial species on the specific medium that is Skim Milk Agar (SMA) medium and Nutrient Agar + Lipid (NAL), then found two types of bacterial isolates that capable to hydrolyze proteins which are isolated B and D and types of bacterial isolates able to hydrolyze the lipid that is bacterial isolates A, B, C, D, E, F, G, H and I. Then made the confirm based on the level of protein hydrolysis of clear zones around colonies of bacteria growing in SMA medium, the greater the clear zone, the higher the level of protein hydrolysis ability of these bacteria.

In order to confirm lipid hydrolysis ability level from each isolate in qualitatively, it based on the presence of red in the NAL medium. The more red the color of colonies on the bottom of the media, the higher the lipid hydrolysis ability of a species of bacteria. While the pale red color of colonies on the basis of the medium, the lower the ability of the species in hydrolyzes lipids. The data results of testing the ability of bacterial species in indigen hydrolyze lipid can be seen in Table 1.1 below.

Table 1.1 Testing Results Data Capabilities Bacterial Isolates hydrolyze proteins and lipids in Qualitative

Bacterial Isolates Code	Hydrolysis Ability Protein
A	—
B	+
C	—
D	+
E	—
F	—
G	—
H	—
I	—

Note:
— = Not able to hydrolyze protein
\+ = Ability to hydrolyzes protein

Bacterial Isolates Code	Hydrolysis Ability Lipid
A	+
B	++
C	++
D	+
E	+++
F	+++
G	+++
H	++
I	+++

Note:
— = Not able to hydrolyze lipid
\+ = Low ability to hydrolyzes lipid
++ = Medium ability to hydrolyzes lipid
+++ = High ability to hydrolyze lipid

Based on Table 1.1 it can be seen that the bacterial isolates that have the ability to hydrolyze proteins by having a transparent color on the bottom of the medium is a bacteria isolates B and D, while isolates of bacteria other than B and D do not have the ability to hydrolyze proteins.

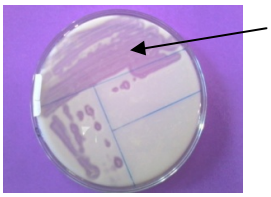

Figure 1.1 Qualitative Ability Protein Hydrolysis by Proteolytic Bacteria in SMA Medium

Note:
a = Transparant color colonies on the bottom of a medium that shows that bacteria are proteolytic, which is able to hydrolyze the protein in the medium school

In qualitative testing the hydrolysis of bacterial isolates that have been found, the bacteria that little red at the bottom of the medium is the type of bacterial isolates with codes A and D. This suggests that the type of bacterial

isolates with that code has the ability to hydrolyze low in lipid. The type of bacterial isolates that have the ability to hydrolyze lipid to the ability of being is to isolate bacteria with code B, C and H. Bacterial isolates with the code E, F, G, and I is the bacterial isolates are able to hydrolyze lipids with high ability. Determination of the ability of hydrolyze lipid qualitatively based on shades of red from the colony at the bottom of the medium (see Figure 1.1). The photos of the testing of each isolate can be seen in Appendix

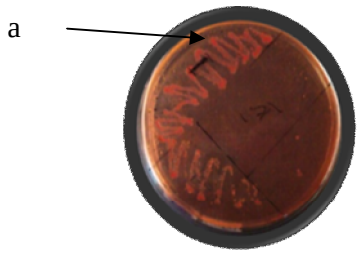

Figure 1.1 Test of Qualitative Ability Lipid Hydrolysis by lipolytic bacteria in the NAL Medium

Note:

a = red colony color on the bottom of a medium that shows that bacteria are lipolytic, which is able to hydrolyze lipid in the medium NAL

This research result shows that there are 9 bacteria isolate that found in the fish flesh. Between these bacterias isolate there are 2 isolate of proteolytic bacteria that can hydrolize protein and 9 isolate of lipolytic bacteria that can hydrolize lipid.

This fact shows that the preservation of this fish have limit preserve time. After preservation, some proteolytic and lipolytic bacterias could contaminated this fish and cause damaged.

4 Conclusion

1. There are 9 isolates of bacteria found in fish samples
2. There are two isolate of proteolytic bacteria in preserved *Rastrelliger canagurta*
3. There are nine isolate of lipolytic bacteria in preserved *Rastrelliger canagurta*.

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