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FAKULTAS TEKNOLOGI PERTANIAN UNIVERSITAS UDAYANA BUKIT JIMBARAN, BADUNG, BALI

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Perkembangan ilmu teknologi pertanian dewasa ini sudah sangat berkembang dikarenakan berbagai aspek kehidupan membutuhkan sentuhan teknologi termasuk dalam pemenuhan terhadap kebutuhan pangan. Oleh karena itu, ilmu teknologi pertanian sudah mengembangkan dirinya ke arah yang tidak terpikirkan sebelumnya. teknologi informasi, robotic bahkan teknologi nano pun tidak melepaskan dirinya dalam berkontribusi memajukan teknologi pertanian. Kedepan tantangan yang dihadapi manusia dalam usaha pemenuhan kebutuhan pangan akan bisa dijawab oleh interkoneksi antara berbagai sub teknologi yang secara konsisten menuju pada efektivitas dan efesiensi yang lebih baik. Untuk itu, kami redaksi sangat membuka diri untuk menyebarluaskan segala hasil penelitian terkait dengan teknologi pertanian, sehingga hasil penelitian semakin dekat dengan para pembaca yang pada akhirnya mampu berperan dalam upaya peningkatan kesejahteraan pertanian dalam arti luas. Mari jadikan jurnal ini sebagai media berbagi dan menyebarkan ilmu yang berguna bagi masyarakat.

Redaksi

## Preservation of Ribbon fish (T richiurus lepturus ) using lactic acid bacteria cultured isolated from wild horse milk

by Ni Nyoman Puspawati

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### Preservation of *Ribbon* fish (*Trichiurus lepturus*) using lactic acid bacteria cultured isolated from wild horse milk

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#### Abstract

The research was aimed to extend storing time of Ribbon Fish in the room temperature, to find out the storing time of Ribon Fish in the room temperature using LAB culture isolated from wild horse milk. Kind of LAB that used was Lactobacillus rhamnosus SKG 15a1 isolated from wild horse milk. This research was designed using factorial randomized block design consist of two factors. First factor was soaking method with four levels; those were 0, 10, 20 and 30 minutes. Second factor was storing time consist of four levels; those were 0, 8, 16 and 24 hours. Each treatment was repeated twice so yield 32 experimental units. The results showed that interaction between the two treatments affect the value of pH, totally microbe and totally lactic acid bacteria of Ribbon fish. The treatment of soaking with LAB culture and storing time influenced the protein contents and also affected the sensory quality, especially smells, appearance of eyes, gills color, elasticity of meat and overall acceptance of the Ribbon Fish. Based on the analysis of all variable, the best treatments combination was soaking with LAB culture of 10 minutes and 8 hours storing time with objective characteristics of pH value was 6.81; totally microbe 6.35 log cfu/g; totally lactic acid bacteria 5.21 log cfu/g and subjective characteristics i.e. fresh smells, clear eyes looking, red light fish gills color, elastic meat and fresh overall acceptance.

Key words: Preservation, soaking, storing time, Ribbon fish (Trichiurus lepturus), culture of Lactobacillus rhamnosus SKG 15a1

#### INTRODUCTION

Ribbon fish (Trichiurus lepturus) is a fish of high economic value and large enough in area waters obtained Badung. Fish is a food that easily damaged, especially in a fresh condition will be quickly damaged so the quality is low. So far the presservation and processing of fish canning salting and manufacture of fish meal. Processing is quite simple and easy is by salting, but it tastes salty then the amount to be consumed relatively little. To overcome these preservation techniques it is necessary to find another alternative to use culture Lactic Acid Bacteria (LAB). Ribbon fish of fresh have a relatively short shelf life because the fish have high water content (80%) and near neutral pH of the body so it is a good medium for the growth of spoilage bacteria and other microorganisms. LAB is selected Lactobacillus rhamnosus 15a1 SKG because it produces lactic acid in the fermentation process that can be exploited to suppress the growth of spoilage bacteria that is expected to preserve Ribbon fish at room temperature.

#### METHODS

#### **Materials Research**

Materials used in this study consisted of *Ribbon* fish (trichiurus lepturus) obtained dati fish auction (TPA) in Kedonganan, Jimbaran, Bali. Lactic Acid Bacteria culture (LAB) SKG 15a1 *Lactobacillus rhamnosus* strains isolated from wild horse milk obtained from the results of previous studies (Riris, 2008).

Chemicals and media used for the analysis is crystal violet, Lugol, 95% alcohol, safranin dye, hydrogen peroxide (H2O2) 3%, medium de Man Rogosa Sharp (MRS) broth, de Man Rogosa Sharp medium (MRS) agar, milk skim, glucose, a solution of BCP (Bromo Cresol Purple), NaOH, NaCl 0.85%, medium Plate Count Agar, aquades, 4 and 7 buffer solution.

#### Implementation Research Refreshment Isolates LAB

LAB isolates in a frozen state (-80°C) in the loop and grab a needle inserted into a test tube containing MRSB as much as 5 ml, then incubated for 48 h at 37°C. Positive results are indicated by the onset of turbidity in the tubes.

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#### Confirmation of Isolates LAB

Prior to this research, LAB culture confirmed in advance to find out the nature and type of culture used LAB. Confirmation includes morphological cultures were tested with Gram staining besides the physiological properties of the culture was also determined that include catalase test, and the ability to produce CO<sub>2</sub> gas from glucose.

#### Work Culture Making LAB

Making culture work carried out by following the steps as follows: first stage, LAB inoculum from stock culture as much as 3-4 needle loop is taken and transferred in 5 ml MRSB. Subsequently incubated at 37°C for 48 hours. The second stage is the stage before making the parent culture by creating media 10 grams of skim milk reconstituted with aquades to 100 ml and then pasteurized at a temperature of 70 -80 ° C for 30 minutes. Furthermore, inoculated 0.1 ml of inoculum from the first stage into the skim milk medium and incubated at 37°C for 48 hours. The third stage is the stage of manufacture of culture between the parent as much as 0.1 ml of culture medim was inoculated in 100 ml of skim milk and then incubated at 37°C at for 48 hours. The fourth stage is the creation of work culture in skim milk medium 100 grams plus 30 grams of glucose dissolved in aquades to 1 liter and then pasteurized. A total of 5 ml of culture medium between the inoculated and incubated at 37°C for 48 hours. Work culture is used to maintain the freshness of the Ribbon fish. In the fourth stage is also carried out counting the total LAB culture of work that will be used. This test aims to determine the initial number of bacteria used in the process of fish layur using dispersive method with a specific medium MRS agar (Hadiwiyoto, 1982).

#### Preservation of Ribbon Fish

Cultures of *L. rhamnosus* used SKG 15a1 containing living cells 108 cfu / ml. Fish used is fresh fish catch of fishermen who bought in TPI Kedonganan. At this stage of preservation, the work culture of *L. rhamnosus* SKG 15a1 put in a sterile container and fish that have been cleaned *layur* incorporated into it until all the fish submerged. At this stage *layur* without preservation of fish culture and working as a control immersion treatment of fish in the work culture for 10, 20 and 30 minutes later carried out of storage at room temperature. Observations and analysis was done at 0, 8, 16 and 24 hours. Observations on the hour to-0 when the sample is being subjected in the laboratory. Analysis

conducted a chemical analysis (pH), microbiological (total microbial and total LAB) and sensory evaluation (odor, appearance of eyes and gills, elasticity and overall acceptance).

#### Observed variables

Variables that were observed in *layur* fish preservation include total microbial analysis, total lactic acid bacteria (LAB), acidity (pH) and sensory evaluation.

#### RESULTS AND DISCUSSION

#### **LAB Culture Confirmation**

Confirmation is based on LAB culture morphological and physiological traits, which form isolates by Gram stain obtained results similar to Figure 1. From the results of morphological observation with Gram, *L. rhamnosus* SKG 15a1 provide color purple so-called gram-positive bacteria with a single trunk. These isolates also did not produce gas and has no catalase enzyme activity so that isolates of *L. rhamnosus* are homofermentative 15a1 SKG.

#### **Total Microbes**

The results of diversity analysis showed that the interaction between treatment, long soaking treatment with LAB culture (R) treatment and storage at room temperature (P) has very significant (P <0.01) to the total microbial *layur* fish as shown in Figure 2.

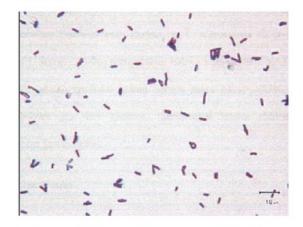
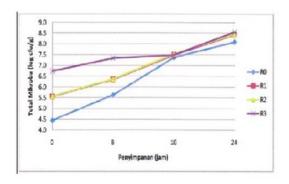


Figure 1. Cultures of *L. rhamnosus* SKG 15a1 (magnification 1000x)

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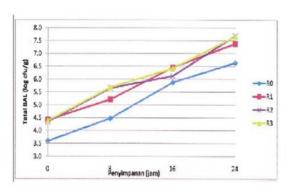


**Figure 2.** Total microbes in *Ribbon* fish at long immersion treatment with LAB culture and treatment of storage at room temperature

The longer soaking time with LAB cultures and storage time the more total microbes will increase. From the above data in mind that the total microbial Ribbon fish lowest on treatment without immersion (control) that is equal to 4.45 log cfu/g of fish, after storage for 8 hours, total microbial increase by 1 log cycle that is to be 5.63 log cfu/g, to 5 microbes after storage for 16 hours that is equal to 7.37 log cfu/g and after 24 h to 8.09 log cfu/gram. Calculated as the total number of microbes is microbes are naturally present in fish plus the number of LAB into the fish. So the longer soaking time and storage time the number of microbes that enter the fish will be even greater. According to Jeanie et al (1997), handling the fish after being caught like a fish soaked in sea water is dirty, do not keep the container and environmental sanitation, the dismantling of fish that are too long and the unavailability of clean water to wash any container of fish will increase the number of microbes in fish. Factors that support microbial growth is the availability of nutrients, water, temperature, pH, oxygen and oxidation reduction potential, presence of inhibitors and the presence of other microorganisms.

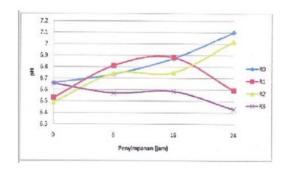
#### **Total Lactic Acid Bacteria**

The results of diversity analysis showed that the interaction between treatment effect not significant (P> 0.05), while the long soaking treatment with LAB culture (R) treatment and storage at room temperature (P) significant effect (P <0.01) on total LAB layur fish as shown in Figure 3. The longer soaking with the more LAB cultured, LAB amount of penetration into the fish. LAB species that dominated during the fermentation of fish are naturally Leuconostoc sp, Lactobacillus sp, Pediococcus sp and Streptococcus sp (Aryanta, 1994).



**Figure 3.** Total lactic acid bacteria in *Ribbon* fish at long immersion treatment with *LAB* culture and treatment of storage at room temperature

The longer the storage time would cause the number of LAB has evolved into much more so that it can inhibit the growth of spoilage bacteria. Sutoyo *et al* (1998) said that *Lactobacillus sp.* has exponent-tial growth phase after 2 hours of incubation, stationary growth phase after 16 to 24 hours of incubation and the final phase of cell death after 32 hours incubation. Increasing the number of total LAB is also followed by a decrease in pH value in the treatment.



**Figure 4.** Changes in pH value in the treatment of *Ribbon* fish long immersion treatment with culture of LAB and storage at room temperature

#### The degree of acidity (pH)

The results of diversity analysis showed that the interaction between treatment, long soaking treatment with LAB culture (R) treatment and storage at room temperature (P) has very significant (P < 0.01) to pH layur fish as shown in Figure 4. The longer soaking time in the LAB culture pH value of Ribbon fish be on the downside. This is because the formation of acids that can lower the pH due to the activity of LAB were added and the soaking time is

longer and thus affects the pH value becomes lower *Ribbon* fish. Similarly the longer the storage, pH decreased *layur* fish resulting from the formation of lactic acid as a result of metabolism of simple sugars by LAB.

#### Sensory evaluation

Sensory evaluation carried out on Ribbon fish including odor, eyes appearance, gill color, texture and overall acceptance by using test scores. The average value of the results of sensory evaluation on the long immersion treatment with culture of LAB (R) and storage at room temperature (P) can be seen in Table 1. During storage of fish odor scores decreased from fresh to normal criteria. Odor scores decreased microbial activity allegedly caused the break down proteins that produce volatile compounds such as ammonia, methylamine, etc. (Jeani et al., 1997). During storage of fish eye

appearance scores decreased from normal to clear criteria. According Hadiwiyoto (1993), changes in the freshness of the fish will cause a marked change in the brightness of the eyelets. During storage of fish gill color score decreased from bright red criteria until light brown. Gill is a blood center in taking oxygen from the water. Fish deaths cause the role of blood (hemoglobin) to stop, even reverse the blood can be oxidized so that the color changed to dark red. During storage of fish flesh firmness score decreased from chewy to usual criteria. Microbiological damage and autolysis in fish will cause the destruction of fish meat. In the fresh fish, fish meat chewy, not to lose fluid so that the fish still looks wet and a few hours after the dead fish, meat, fish become stiff, lose the freshness, the onset of the liquid as droplets of water that flows to the outside and chewy texture of the meat will lose.

**Table 1**The average value of the results of sensory evaluation in *Ribbon* fish

Treatments	Odor	Eye	Fish gill color	Flesh firmness	Overall
R0P0	5,00a	5,00a	5,00a	5,00a	5,00a
R0P1	4,07b	4,07bc	3,27d	4,07b	4,07bc
R0P2	3,47d	3,27e	1,93e	3,27d	3,27e
R0P3	2,73e	2,53f	1,47f	2,47e	2,47f
R1P0	5,00a	5,00a	5,00a	5,00a	5,00a
R1P1	4,87a	4,73a	4,73ab	4,73a	4,73a
R1P2	4,67a	4,00bc	4,47bc	3,67c	4,00bc
R1P3	3,87bc	3,73cd	3,47d	3,40cd	3,73cd
R2P0	5,00a	5,00a	5,00a	5,00a	5,00a
R2P1	4,73a	4,80a	4,80ab	4,80a	4,80a
R2P2	3,87bc	4,13b	4,13c	4,13b	4,13b
R2P3	3,07e	3,60d	3,33d	3,33cd	3,60d
R3P0	5,00a	5,00a	5,00a	5,00a	5,00a
R3P1	4,20b	4,73a	4,73ab	4,73a	4,73a
R3P2	3,67cd	4,13b	4,13c	4,13b	4,13b
R3P3	2,80e	3,53de	3,40d	3,47cd	3,53de

#### CONCLUSION

Based on the results of research on preservation of *Ribbon* fish (*Trichiurus lepturus*) using LAB cultures from the wild horse milk, it can be concluded that the use of LAB cultures isolated from wild horse milk can extend the shelf life of fish at room temperature *layur*. *Layur* fish shelf life at room temperature can be extended up to 8 hours with LAB culture immersion for 10 minutes with the objective characteristics of the total microbial

6.3544 log cfu/g, total LAB 5.2131 log cfu/g, pH 6.81 whereas subjective characteristic odor fresh, clear eyes, bright red gills color, chewy texture and overall acceptance fresh.

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