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The Influence of Culture Age and Soaking Time Range with Filtrate Lactobacillus Acidophilus toward the Number of Coliform Bacteria in Swordfish (auxis rochei) Stew

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Abstract
This research was conducted to know the effect of culture age and soaking time range in the filtrate culture of Lactobacillus acidophilus in MRS medium towards total coliform bacteria in swordfish stew after bones were taken out from within water. This research was an experimental research conducted in the Laboratory of Food of Chemical Research Center of LIPI Bandung using completely randomized design with three replications. L. acidophilus culture age used were 10, 14, and 18 hours with a range of soaking time 30, 60 and 90 minutes. The parameters used were total coliform bacteria in swordfish stew with MPN (Most Probable Number). The results showed that treatment with a variety of culture 10 hours of immersion time in 90 minutes (MPN 0.61); culture age 14 hours of immersion time in 30,60 and 90 minutes (MPN 0.36, 0.24, 0.12) and 18 hours of immersion time in 30,60 and 90 minutes (MPN 0.49 and 0) can inhibitory the number Coliform bacteria. The culture age 18 hours of immersion time in 90 minutes inhibitory Coliform bacteria up to MPN 0 with control MPN 2.63 without soaking time or 2 log cycle inhibitory Coliform bacteria group. This treatment proved that the culture filtrate L. acidophilus can be used as bio-preservative in swordfish stew.
Keywords: lactobacillus acidophilus, MRS, swordfish, coliform

1. Introduction

Lactic acid bacteria (LAB) in metabolism produces lactic acid as the main products and manufactures a variety of other potential compounds as antimicrobials such as hydrogen peroxide, diasetic, organic acids, and bacteriocins. Lactic acid produced by LAB can inhibit the growth of unwanted microbes in food. While bacteriocins are protein compounds that have a bactericidal effect against other microorganisms. Bacteriocins produced by BAL are potential to be used as a natural food preservative (Ray, 1994).

Lactobacillus acidophilus, one of the BAL which stand to high temperatures (thermophilic facultative 60-80 ° C) and resistant to acid pH of 4 to 4.6 and alkaline pH 7, is a normal flora in the gut, which is very important in providing the defense of the gastrointestinal tract in a way inhibit microbial colonization of pathogens (Subijanto & Rahuh, 2005). L. acidophilus have criteria as probiotic acidophilus, this is based on the resistance of isolates to acidic conditions and a variety of antibiotics, as well as the rate of growth, acid production and its ability to suppress the growth of pathogenic enteric bacteria (Purwadhani, et al. 2007).

Swordfish is the object of a commercial tuna to be exported as wood fish known as arabushi. In the processing of tuna into arabushi, fish boiled then drained, then soaked in water and plucked thorns. At this stage the fish meat are particularly vulnerable to contaminants (BSN, 2009a). Fish meat is a suitable medium for bacterial growth. Bacterial growth was supported by protein content and high water and fish meat pH near to neutral (Adawyah, 2007).

Coliform is a group of bacteria that often contaminate food products. If there is coliform in food, it means that food has been contaminated by human or animal feces. Presence of coliform bacteria is an indicator to determine if food contaminated with organic waste material, especially fecal material. Therefore, it is possible that food is likely to contain pathogenic bacteria from sewage. Bacterial genus Salmonella, Vibrio, and Clostridium are several types of pathogenic bacteria that can contaminate fishery products (Frazier and Westhoff, 1988). Water contamination by Escherichia coli can also cause contamination of fishery products.

Security control of fishery products by the concept of Hazard Analysis Critical Control Point (HACCP) is a requirement of FAO and internationally. The concept is based on the proficiency level of critical control points at

each stage of production. In the process of arabushi, a critical point will be at the revocation of the bones and spines soaked in raw water. It is vulnerable to contamination by bacteria, making it less hygienic. To prevent contamination, we need a processing technique and the proper and safe preservation. (Ray, 2004 & Usmiati, 2009).
L. acidophilus culture filtrate containing antimicrobial compounds that can inhibit / kill contaminants bacteria (Karaoglu et al, 2002., Kusmiati & Malik, 2002). Appropriate age culture is important to know to get filtrate containing the antibacterial. Besides, it needs to know the exact timeframe of immersion so that the inhibition of contaminants bacterial are reached.
Some of the studies support the implementation of this research are for examples: research done by Amin and Leksono (2001), a decrease of microbial growth in smoked fish jambal siam (Pangasius sutchi) that has been preserved by ensiling; Ibrahim and Salha (2009), the metabolite of L. acidophilus effectively inhibit the growth of S. aureus, E. coli and Yeast thus it improves the quality of tilapia fish (Oreochromis niloticus). In addition, Santoso, (2009) utilizing the production of bacteriocins L. Ed plantarum 22 as a preservative in products such as fish fly and shrimp paste can reduce bacterial contaminants during storage.
The objective of this research is to determine the influence of age and time L.acidophilus culture immersion to total coliform bacteria group on the boiled meat tuna (A. rochei).

2. Materials and Methods

2.1 Materials and Equipment

Tools used in this study were petri dishes, test tubes, volume pipettes (Eppendorf), erlenmeyer, ose, incubator (Memmert), autoclave (HL 36 Ae), a spectrophotometer (Hitachi U-2800), pH-meter (CyberScan 510 pc), sentrifugator (Eppendorf 5804 A), oven (Memmert), centrifuge tubes, test tube shaker, analytic scales (AA 200), 0.45 micrometer size Millipore membrane, UV boxes, plastic, scissors, tweezers, durham tube, cool-box, cotton, plastic, laminar air flow (Labconco) square baking dish.
This study uses Tuna as the object of the research obtained from Caringin Market in Bandung, Lactobacillus acidophilus obtained from the Microbiology Laboratory of ITB, deMan Rogosa Sharp (MRS) Oxoid CM 359, Nutrient Agar (NA) Oxoid CM 3B, physiological saline, distilled water, Brilliant Green Lactose Bile Broth (BGLB) Oxoid CM 31B, Lactose Broth (LB) Oxoid CM 317B, 70% alcohol.

2.2 Research Method Phase 1

Making Growth curves of Lactobacillus acidophilus (Cappucino and Sherman, 2005).
Pure culture L. acidophilus activated 3 times. The first activation was inoculated 1 ose of pure L. acidophilus into 10 mL MRS broth and then incubated at 37 ° C for 24 hours. Second activation was inoculated with 1 mL of first activation culture into 9 mL MRS broth and then incubated at 37 ° C for 24 hours. Activation third with five inoculated second activation mL culture into 45 ml MRS broth and then incubated at 37 ° C for 24 hours.
The third culture activation results taken 15 mL and put into Erlenmeyer flask containing 135 mL of sterile MRS broth medium and ready to measure the growth of the bacteria with an interval of every 2 hours. First as t = 0 then the sequence t = 2, t = 4 and so on until t = 24. Measurement of bacterial growth in the 2 methods, namely: indirect measurement or OD ("Optical Density") and direct measurements or DC ("Direct Count"). OD measurements done by looking at the density of bacteria by using a spectrophotometer with a wavelength of 600 nm, while the DC measurement is done by plating duplicate in MRS agar medium. Colony counting is done by making serial dilutions in physiological NaCl.
One mL samples depicted in a series of dilutions to 10^{-1} – 10^{-9}, each containing 9 ml physiological NaCl. Then plating was done and then incubated at 37 ° C for 24-48 hours. Data was made in the growth curve. X axis is the OD value and the number of bacterial cells, and the Y axis shows the time of incubation.

2.3 Research Method Phase II

Tuna fish and fish conditions. Fresh tuna weeded, gills and entrails removed, then washed with water and then boiled for 30 minutes at a temperature of 80 C. Then, fish is drained until cool and put in a container of water wells while prickly plucked using tweezers, then drained for 30 minutes in a dry place at room temperature.
Filtrate L.acidophilus (Deegan et al, 2005; Ogunbanwo et al, 2003).
L. acidophilus obtained from the Laboratory of ITB, cultured in media deMan Rogosa Sharp (MRS) Oxoid CM 359 at a temperature of 37 °C for 10 hours, 14 hours and 18 hours. The culture was centrifuged at a speed of 6000 rpm for 15 min at 4 ° C, then filtered with Millipore membranes size 0:45 micrometer. The filtrate pH was measured using a pH meter, and the filtrate was exposed under UV light for 40 minutes.

Soaking the fish with the filtrate (Amin and Leksono, 2001).
Tuna meat put in rectangle pan containing 200 ml of culture filtrate L. acidophilus with four standard age of culture (control 0, 10, 14, and 18 hours), each pH measured. The fish meat soaked for 0, 30, 60, 90 minutes. Fish meat that has been treated was drained in a dry place at room temperature for 30 minutes.
The calculation of the number of coliform group (BSN, 2009b)
Total Coliform Group was calculated by taking 25 g sample of fish flesh put in 250 ml physiological NaCl 0.9% (10^0) and then diluted with 0.9% NaCl physiological and tested MPN (Most Probable Number). Test probe conducted sample dilution, the last three dilution in each sample put as many as 1 ml in 3 pieces test tube containing 9 ml Lactose Bbroth (LB) medium Oxoid CM 317B which contains a durham tube. Then incubated at 37 ° C for 24-48 hours and observed the formation of gas occurs. Every tube of probe test which positively (contain formation of gas) conducted further tests to be moved 1 ose of each into a tube containing 2 ml of Brilliant Green Lactose Bile Broth (BGLB) Oxoid CM 31B which contains a durham tube. Then incubated at 37 ° C for 24-48 hours. The test results are positive further sign by the formation of gas in durham tube, while to know the results of the final calculation is read by using the MPN table (BSN, 2009b).

3. Research Design

This study was conducted on an experimental basis with a completely randomized design factorial 4 x 4, the first factor consisted of four standard age of culture (0, 10, 14, and 18 hours), each pH was observed, the second factor consists of four immersion period (0 as controls of, 30, 60, 90 minutes) of which are repeated 3 times. Parameters observed is the number of coliform bacteria group found in tuna meat stew with MPN method (Most Probable Number). Data processed by ANAVA (Analysis of Variance) and if the results are significantly different then followed by Duncan's multiple range test.

4. Results and Discussion

4.1 Phase 1 of Research

L.acidophilus Growth Curve is presented below.

Figure 1. Curve of L.acidophilus Growth in MRS Medium

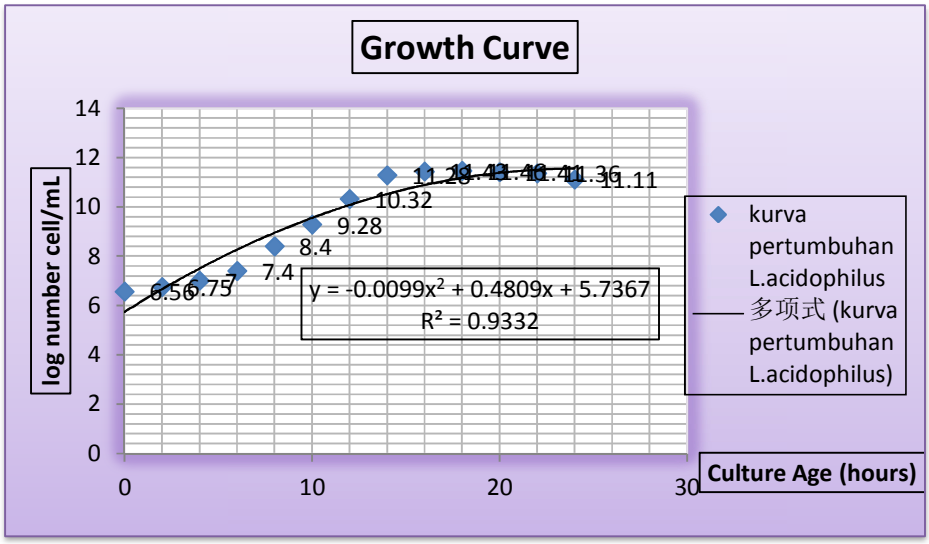
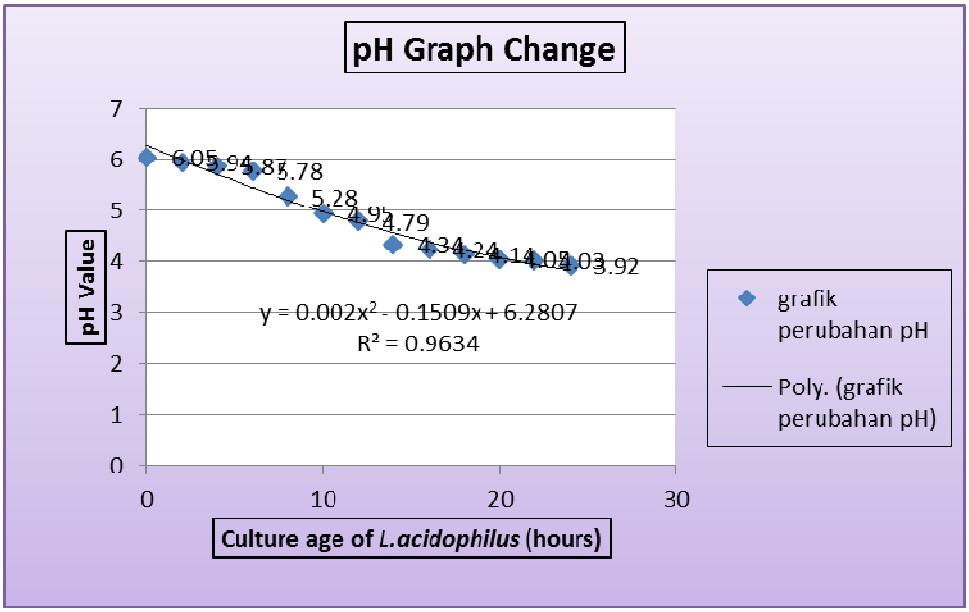


Figure 1 shows that L.acidophilus through a phase of adaptation (lag) in 0 to 2 hours, this phase describes that there is no significant growth population due to cell changes chemical composition and the size and intracellular substances increased that are ready to split themselves. At 2^{nd} – 16^{th} hours L.acidophilus experiencing the next phase, namely the phase of exponential or logarithmic (log), this phase describes the cells divide at a constant rate, the mass is doubled with the same rate, metabolic activity is constant, and the growth is balanced. At the 16^{th} – 20^{th} hour L.acidophilus experiencing the next phase or remain stationary phase, this phase describes the accumulation of metabolites results of activity of cellular metabolism and nutrient content began to run out, resulting in nutrient

competition so that some cells die and others continue to grow so that the number of cells to be relatively constant . At the 20th hours cells experienced death phase, this phase describes the dead cells is faster than the formation of new cells, a high rate of mortality. Graph of L.acidophilus pH culture changes is shown below.

Figure 2. Graphic of pH L.acidophilus Growth



Based on the changes of pH in the growth of L.acidophilus, so the curve of the equation Y = 0.002 X^2 + 0.150 X + 6.280 with the X axis showing the age of the culture and the Y axis shows the pH value. The growth of L.acidophilus, the age of culture is inversely proportional to pH, with the increasing age of the culture pH decreased. This is because L.acidophilus is a homofermentative lactic acid bacteria that produce lactic acid as the primary metabolite.

4.2 The Effect of Different Age Culture and Range Immersion Time with L.acidophilus Filtrate to Total Coliform Group in Tuna Fish Stew Meat.

The Effect of the age culture and immersion time of L. acidophilus with culture filtrate to total coliform group can be seen below.

Figure 3. The Influence of Culture Age and The Immersion Time Range with The filtrate of Lactobacillus acidophilus Against Coliform Bacterial in Swordfish Stew

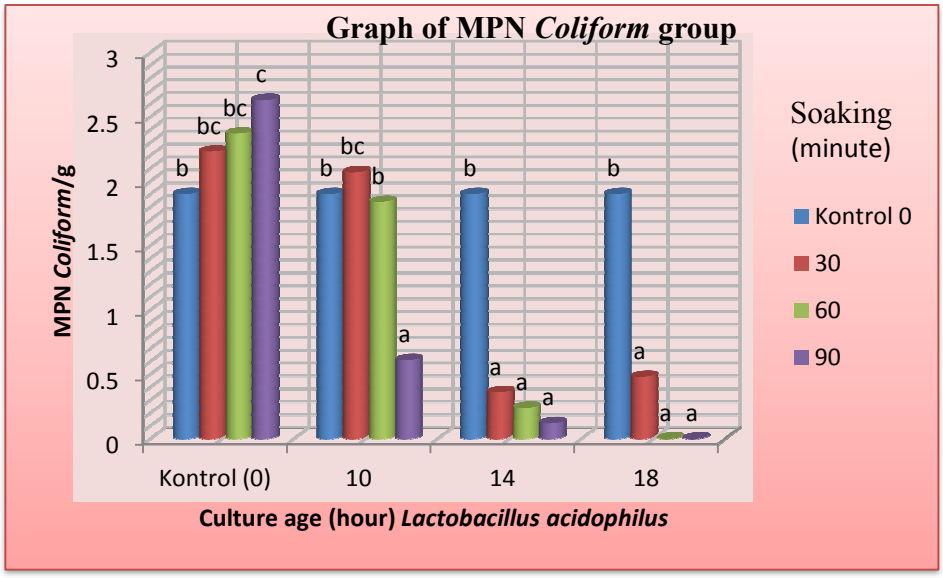


Figure 3 shows that the soaking with culture filtrate L.acidophilus age (10, 14 and 18 hours) and soaking time (30, 60 and 90 min) can inhibit the number of coliform found in tuna meat stew. Culture filtrate L.acidophilus age (10, 14 and 18 hours) have different abilities to inhibit the amount of coliform found in tuna meat stew. Culture filtrate L. acidophilus age 18 hours have a great effect in inhibiting the number of coliform compared to culture filtrate ages 14 and 10 hours. Culture filtrate of L. acidophilus age 14 hours have a greater influence to inhibit the number of coliform culture filtrate compared to the age of 10 days, while the control without an increase in the number of filtrate coliform bacteria group. The effects of various age-related culture filtrate content and pH (age 10 hour culture filtrate produced by pH 4.95, age of culture 14 hours produces filtrate with pH 4:34 and 18 hours old culture filtrate produced by pH 4.14.

Soaking time of stew meat tuna in the filtrate effect on the inhibition of the number of coliform. Culture at age 10 hours, duration of immersion influence the inhibitory of Coliform number is 60 and 90 minutes, ages 14 and 18 hours of culture, the time span that influence the number of Coliform inhibition is 30, 60 and 90 minutes, while the control fish stew without soaking in the culture filtrate of L.acidophilus at any age culture does not inhibit Coliform or relatively similar. Soaking time range associated with the ability of the filtrate in inhibiting the growth of the number of coliform.

Based on the analysis of variance showed that the interaction of age culture and culture immersion period have a significant effect ($P < 0.01$) to total coliform bacteria in tuna meat stew. Based on the results of Duncan's multiple range test showed that treatment on different ages culture and cultures soaking period, will give different result to the inhibitory of coliform bacteria group on the boiled meat tuna. Culture of 10 hours age (pH 4.95) 90 min immersion (MPN 0.61) and immersion treatment with culture filtrate age 14 hours (pH4.34) (MPN 0:36, 0.24, 0.12) and 18 hours (pH 4.14) (MPN 0:49 and 0) soaking time 30, 60 and 90 minutes has the same effect ($P < 0.01$) to the inhibition of the growth of coliform bacteria group in the tuna meat stew. It means that there is inhibition of the growth of coliform bacteria group of aged culture filtrate immersion results 10 hours in 90 minutes, age cultures 14 hours for 30,60,90 minutes and age of culture 18 hours for 30,60,90 minutes compared to the fish stew without soaking in the filtrate L.acidophilus log value of 2.63. Meaning that there is the inhibitory of 2 log cycles of Coliform bacteria group. This treatment proved that the culture filtrate L. acidophilus can be used as biopreservative the tuna meat stew.

The age of L.acidophilus culture during the exponential growth phase produces lactic acid as the primary metabolite. The production of bacterial metabolites is influenced by factors such as the availability of nutrients as the energy source that MRS medium, optimum growth temperature 37 ° C and the condition of facultative an aerobe. (Banwart, 1989).

Soaking time relates to the timeframe required by the various age culture filtrate L.acidophilus for penetration into the fish stew that is the time to reach equilibrium between the same isoelectric point of pH and filtrate pH tuna stew that filtrate could inhibit coliform bacteria group, the culture filtrate of L.acidophilus pH 4.95 at age 10 hours, age 14 pH 4.34, age 18 hours at pH 4.14. 10 hours culture filtrate, it will take immersion time (up to 90 minutes) to inhibit the growth of coliform bacteria group. Age cultures at least 14 hours or up to 18 hours, it will take a minimum of 30 minutes of immersion or a maximum of 90 minutes to inhibit the growth of coliform bacteria.

Filtrate L.acidophilus can affect the amount of coliform in the stew meat tuna because L.acidophilus produce lactic acid as the primary metabolite, accompanied by the production of H_2O_2, diasetil, CO_2, and bacteriocins. Lactic acid produced by L.acidophilus decrease the pH of the filtrate and reduce the number of coliform group in tuna meat stew. Coliform group is classified as sensitive to acid (pH optimum 6-7) so that the acid treatment will affect the numbers. Mechanism of action occurs because the lactic acid in dissociated form can penetrate the cell membrane. In addition, being able to reduce the pH and the situation will disrupt the activity of the enzyme so that the cells can not perform metabolic activities. This is consistent with the statement by Calcioglu, et al (2002) in Rosyidi, et al (2005) that organic acids treatment such as acetic acid and lactic acid can decrease the amount of coliform and pathogens negative Gram 1 to 3 log CFU/cm2.

Besides lactic acid, L.acidophilus produces a compound that is able to inhibit other microorganisms such as H_2O_2, diasetil, and bacteriocins (Ray, 2004). Bacteriocins produced by lactic acid bacteria in the synthesis medium (MRS) is able to maintain the freshness of the meat. Bacteriocins are the secondary metabolites of BAL are synthesized in the mid phase logarithmic to stationary phase has bactericidal characteristics (Santoso, 2009). Lactobacillus can also produce H_2O_2 due to the presence of oxygen and function as an antibacterial that can cause the inhibition of the growth of other microorganisms (Ray, 2004). In addition, hydrogen peroxide able to kill bacteria forming the anaerobic spore (Desrosier, 1988).

5. Conclusion

The immersion treatment to swordfish stew at the age of culture filtrate 10 hours in 90 minutes (MPN 0.61), age of culture 14 hours for 30,60,90 minutes (MPN 0:36, 0.24, 0.12) and age of the culture 18 hours during 30,60,90 min (MPN 0:49 and 0) is able to inhibit the growth of coliform bacteria, compared to stew fish without soaking in the filtrate L.acidophilus log value 2.63. Meaning that there is a 2 log cycle inhibition of Coliform bacteria group. This treatment proved that the culture filtrate L. acidophilus can be used as biopreservative in the tuna meat stew.

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