

**THE INFLUENCE OF GIVING VARIOUS CONCENTRATIONS AND METHOD OF INOCULUM *Lactobacillus acidophilus* ACCORDING TO IMMERSION TIME FOR TOTAL *Escherichia coli* IN SWORDFISH STEW (*Auxis rochei*)**

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**ABSTRACT**

Giving of various concentrations and method of inoculum *Lactobacillus acidophilus* i.e preventive, curative and simultaneous according to immersion time for total *Escherichia coli* in swordfish stew (*Auxis rochei*) has been performed experimentally. The result showed that the treatment of inoculum *L. acidophilus* with  $10^{11}$  cfu/ml concentration according immersion time of 90 minutes gave the best result for the absence of *E. coli*, while reduction of total *E. coli* can be done by using preventive, curative and simultaneous method.

**Keyword :** *Lactobacillus acidophilus*, *Escherichia coli*, *The method of inoculum*, *Auxis rochei*

**INTRODUCTION**

Use of chemicals as a food preservative has been circulated widely in the community. Chemicals are still question its safety for human consumption that is nitrite, sulphite, rhodamine, borax and formaldehyde. With consideration of the food consumed is safe and healthy, then more people like foods that are not chemically preserved, but preserved naturally (1).

Microorganisms are popularly used as an agent biopreservatif are a group of bacteria of the genus *Lactobacillus*, which are groups of lactic acid bacteria (LAB). BAL is often used for food preservation because it is able to produce organic acids lactic acid and form a kind of antimicrobial compounds (bacteriocin) (2). These bacteria are often found in fermented foods, processed fish products, meat, milk and fruits (3).

*Lactobacillus* potentially be used for preservation, among others, are *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus bulgaricus* (4,5) and *Lactobacillus acidophilus* (6). *L. acidophilus* known to produce natural antibacterial substances that can inhibit growth of 27 types of bacteria, 11 of which are pathogenic bacteria (7). Prevention of decay process in fish can be done by various preservation methods and processing quick and accurate for most of

the fish produced can be utilized (8). Biopreservatif is a way to preserve food products such as meat and its products, so avoid decomposition due to contamination by microbes. One alternative way of preserving fishery products are biological (microbiological) is to add the group of LAB as preservatives (2). This preservation method can be performed by various methods which are preventive, curative or simultaneous. Preventive method of prevention against bacterial contaminants to the granting of BAL, curative method of treatment due to infection by bacterial contaminants to the granting of BAL and simultaneous methods of providing working simultaneously bacterial antagonist (9).

Tuna is one type of fish commonly used in the manufacture of wooden fish / arabushi (10). At the stage of the process of making wooden fish, the tuna should be boiled and plucked thorns and bones. Thorn and bone removal process is performed in water immersion, so that there are a number of bacterial contaminants feared (11). The process of decline in quality in fish due to chemical reactions that the enzyme activity and biological reactions of the activity of microorganisms (12,13). Microorganism activity is caused by millions of bacteria that cause spoilage of fish found throughout the body especially on the skin, gills and intestines. This type of bacteria commonly found in fish such as *Achromobacter*, *Pseudomonas*, *Flavobacter*, *Micrococcus*, and *Bacillus* (14). Pollution of waters by *Escherichia coli* can also cause contamination of fishery products. The presence of microbial pathogens on fresh fish shown to affect human health.

Various ways that can be done to overcome the problem of contamination of pathogenic bacteria, which are soaking fishery products in BAL media in preventive, curative and simultaneous. *L. acidophilus* density on the swordfish is influenced by the high initial concentration and dipping time (15). During immersion, *L. acidophilus* will experience the growth that occurred in the number of cells that exceed the amount of initial inoculum. Appropriate bacterial growth cycle, *L. acidophilus* will experience rapid growth (log phase), reaching the maximum number of cells (stationary phase), then the growth rate will decline and eventually stopped (death phase) (16). During immersion in addition to an increase in the number of *L. acidophilus*, also an increase in the number of

bacteria *E. coli*. Increasing the number of bacteria will decrease the effectiveness of antimicrobial compound produced by *L. acidophilus*. Therefore, please be aware and long soaking concentration effective to inhibit bacterial growth for *E. coli*.

## **MATERIAL AND METHODS**

### **Bacterial strains and culture conditions.**

Bacterial culture *L. acidophilus* collection of Microbiology Laboratory of Biological Sciences, Bandung Institute of Technology and culture *E. coli* ATCC 10 536 BIO FARMA Laboratory collection Bandung. Each bacterium was grown in de Man Rogosa Sharp medium (MRS) Agar (Oxoid CM 359) and media Nutrient Agar (NA) (CM 3B).

**Tuna (*A. rochei*).** Swordfish obtained from Caringin Market in Bandung.

### **Determination of Total *E. coli*.**

Number of *E. coli* are inserted in 500 ml boiled water of 10<sup>8</sup> cfu / ml. First made McFarland standard 1 (cell density of 3x10<sup>8</sup> cfu / ml) by mixing 0.1 ml with 9.9 ml of BaCl<sub>2</sub> H<sub>2</sub>SO<sub>4</sub>. As much as 1 ose bacteria *E. coli* was inoculated in 100 ml of Nutrient Broth and incubated at 37<sup>0</sup>C for 24 hours. Then the bacterial culture was centrifuged at 6000 rpm for 15 minutes at a temperature of 4<sup>0</sup>C (17) to obtain bacterial cells. Furthermore, the cells collected are made in accordance with McFarland turbidity 1 with the addition of physiological NaCl until the turbidity equal to McFarland 1.

### **Determination Concentration Inoculum *L.acidophilus* used for Soaking in Tuna Fish Stew Meat (*A. rochei*).**

Preparation of concentration inoculum *L. acidophilus* 10<sup>11</sup> cfu / ml which is about 10 ml of culture with a concentration 10<sup>11</sup> cfu / ml were centrifuged at 6000 rpm for 15 minutes at a temperature of 4<sup>0</sup>C to obtain a bacterial cell. Subsequently 1 ml of bacterial cells obtained (10<sup>12</sup> cfu / ml) was included in 9 ml physiological NaCl so that the fixed dilution 10<sup>11</sup> cfu / ml, then taken in increments of 1 ml and placed in 500 ml of boiled water (concentration of 2x10<sup>8</sup> cfu / ml). Preparation of inoculum concentration *L. acidophilus* 10<sup>8</sup> cfu / ml of inoculum concentration *L. acidophilus* 10<sup>11</sup> cfu / ml were taken 1 ml and put in 9

ml physiological NaCl so dilution to  $10^{10}$  cfu / ml and made up to dilution  $10^8$  cfu / ml, then take 1 ml and placed in 500 ml of boiled water (concentration of  $2 \times 10^5$  cfu / ml). Preparation of inoculum concentration *L. acidophilus*  $10^6$  cfu / ml of inoculum concentration *L. acidophilus*  $10^8$  cfu / ml were taken 1 ml and put in 9 ml physiological NaCl so pengencerannya to  $10^7$  cfu / ml and made up to dilution  $10^6$  cfu / ml, then take 1 ml and placed in 500 ml of boiled water (concentration of  $2 \times 10^3$  cfu / ml).

### **Tuna Fish Poaching and Immersion.**

First fish gills and the entrails cleaned, then boiled for 30 minutes at 75-80°C. After that, the fish is removed and plucked thorns and bones and then drained. Next step is soaking with inoculum *L. acidophilus* with three methods of preventive, curative and simultaneous.

**Preventive method** is soaked in water boiled fish is first given inoculum *L. acidophilus* with various concentrations of  $10^6$  cfu / ml,  $10^8$  cfu / ml and  $10^{11}$  cfu / ml and time of immersion for 30, 60 and 90 minutes. Then the fish is soaked in boiling water containing a culture of *E. coli*  $10^8$  cfu / ml for 30 minutes.

**Curative method** is fish soaked in water cooking containing cultures of *E. coli*  $10^8$  cfu / ml for 30 minutes and then soaked using inoculum *L. acidophilus* with various concentrations of  $10^6$  cfu / ml,  $10^8$  cfu / ml and  $10^{11}$  cfu / ml and time of immersion for 30, 60 and 90 minutes.

**Simultaneous method** is swordfish marinated using a variety of *L. acidophilus* inoculum concentration of  $10^6$  cfu / ml,  $10^8$  cfu / ml and  $10^{11}$  cfu / ml and the culture of *E. coli*  $10^8$  cfu / ml together with various immersion time of 30, 60 and 90 minutes.

### **Calculating the Most Probable Number Bacteria *E. coli* in Tuna Fish Stew Meat (*A.rochei*) After Immersion In Preventive, Curative and Simultaneous.**

#### **Prediction Test**

A total of 25 grams of fish samples were taken and diluted in test tubes, each containing 250 ml of sterile physiological NaCl ( $10^0$ ) (18). Each 1 ml dilution inserted into 3 tubes containing 9 ml of Lactose Broth tubes that have been placed Durham. Samples were incubated at 37°C for 24 hours. Prediction of a positive test is characterized by the formation of gas in Durham tubes (19).

### Supplementary Test

Each tube in the prediction of a positive test to move a loop of each into a tube containing 2 ml of Brilliant Green Lactose Bile Broth (BGLB) and incubated at 37°C for 24-48 hours to confirm the presence of Coliform. Positive test indicated by turbidity and the formation of gas in Durham tubes (19).

### Advanced Test

Each tube positive BGLB taken one loop and inserted in a tube containing 2 ml of EC Broth and then incubated at 44°C for 24-48 hours to confirm the existence of fecal coli. Positive test indicated by turbidity and the formation of gas in Durham tubes

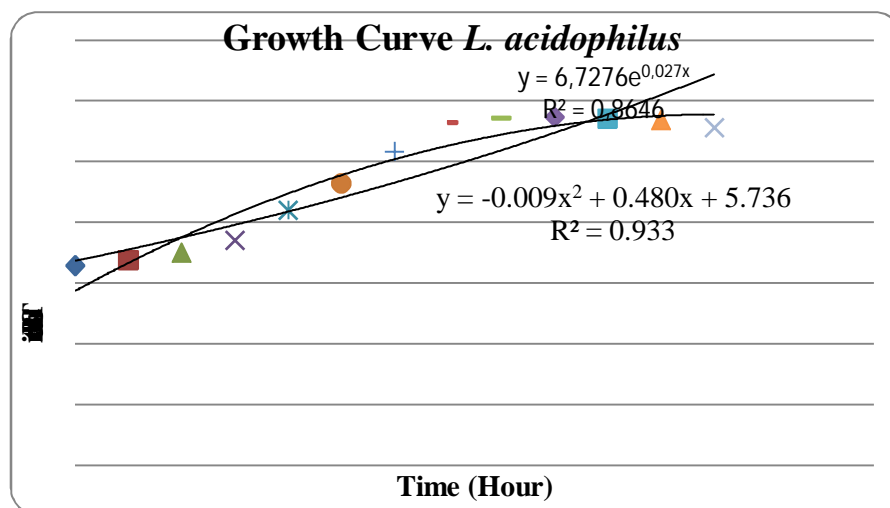
(19).

### Count Bacteria E.coli MPN Method.

The calculation of the number of bacteria E. coli using the MPN (Most Probable Number). From each dilution tube was calculated positive. Combination of numbers is taken from 3 last dilution, where the last digit is zero.

## RESULTS

### Growth Curve L.acidophilus



**Figure 1.** Growth curves of *L. acidophilus* in the medium de Man Rogosa Sharp (MRS)

In the hour -0 to 2<sup>nd</sup> incubation, *L. acidophilus* experiencing adaptation or lag phase where cell division does not occur because this phase is formed from the

adaptation process is complete yet linked to growth media and environmental conditions (20,21).

The next phase is the exponential growth phase or logarithmic (log), which is achieved by *L. acidophilus* which started after hours-2 to achieve maximum growth in the 16 th hour of incubation. In this phase which is the end of log phase, cells begin to divide. Because cell division is exponential equation, then this phase is also called the exponential phase (22). During the phase of logarithmic or exponential phase cells divide continuously in a constant high-speed growth. The population is almost equal and balanced growth in accordance with the activity of metabolic and physiological characteristics (23). While the old culture showing constant growth rate which is the highest cell activity is achieved at the 6 th hour that is used as an early starter for the manufacture of bacterial growth curve *L. acidophilus*.

Stationary phase *L. acidophilus* in MRS media began to appear after an hour of the 16th until the 20<sup>th</sup> hour of incubation. In this phase of the cell population remains because of the number of cells that grow together with the number of dead cells, so the number of bacterial cells is relatively constant. The size of cells in this phase becomes smaller because the cells still divide even though nutrients have started out (20). At the 20<sup>th</sup> till the 24<sup>th</sup> hour incubation *L. acidophilus* has entered a phase of death. During the death phase, bacteria that can live cells decreased in number, almost inverted from what happened during the logarithmic phase. The number of cells that die the longer the more, and the speed of cell death is influenced by nutritional conditions, environment and type of bacteria (20). Endless accumulation of nutrients and products such as acid inhibitors are several factors that affect the mortality of bacteria (23).

## Growth Curve E.coli

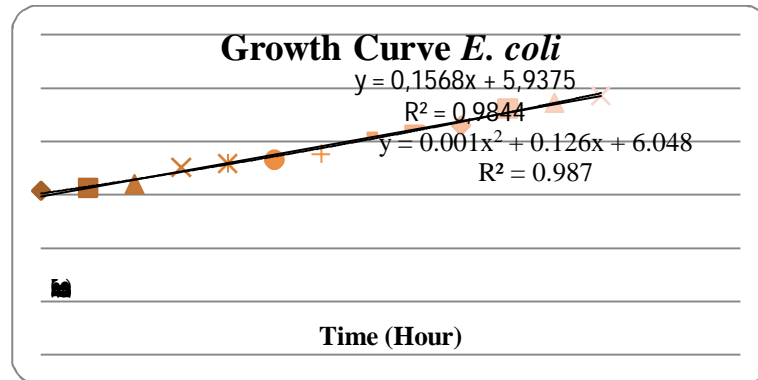


Figure 2. Growth curves of *E. coli* in NA medium (Nutrient Agar)

Growth curve *E. coli* was made to determine the growth stage of *E. coli* and used to determine the generation time of *E. coli* that can be compared with *L.acidophilus* generation time.

Based on Figure 2 shows that *E. coli* still went through a phase of adaptation (lag) is on hour 0 to 4<sup>th</sup> incubation. Just as the growth phase of *L. acidophilus*, a phase of adaptation (lag) *E. coli* cell division has not occurred. The duration of this phase varies, can be fast or slow depending on the speed of adjustment to environmental conditions and growth media (20).

The next phase is the exponential growth phase or logarithmic (log) that began after the 4<sup>th</sup> until the 24<sup>th</sup> hour of incubation. After experiencing a phase of adaptation, the cells begin to divide at a pace that is still low because of recently completed phase of adaptation (20). Later in this phase the cells divide at a constant rate so that the mass is doubled (16).

Generation time (doubling time) *L. acidophilus* and *E. coli*. Based on the results of calculations using formula (16)

$$G = \frac{t}{3,3 \log \left( \frac{b}{B} \right)}$$

G = generation time

t = interval between the measurement of the number of cells in the population at some point in log phase (B) and then again at a later time point (b)

B = initial population

b = population after time t

log = log 10

3.3 = conversion factor to be  $\log_{10} \log_2$

*L. acidophilus* has a generation time of 20.22 minutes, while *E. coli* 36.84 minutes. This shows that the generation time of *L. acidophilus* faster than *E. coli*. Probiotic bacteria such as *L. acidophilus* has the speed of growth and cell division rate is high so that the faster generation time (24). While the generation time of *E. coli* longer due to low speed and the speed of growth of bacterial cell division (25).

**Influence of Various Inoculum Concentration and Method of Giving *L. acidophilus* According to Total Immersion Time *E. coli* in Tuna Fish Stew Meat**

**Tabel 1.** Variance Analysis of Effect of Various Concentrations and Methods Inoculum *L. acidophilus* Giving According to Total Immersion Time *E. coli* in Tuna Fish Stew Meat.

Various source	df	NS	CS	F <sub>count</sub>	F <sub>table</sub>	
					F <sub>0,05</sub>	F <sub>0,01</sub>
Means	1	29383,32	29383,32	167,91		
Immersion time(W)	2	2387,60	1193,798	6,822**	3,26	5,25
Concentration <i>L. acidophilus</i> (K)	3	70193,44	23397,82	133,71**	2,87	4,38
Immersion Method Immersion (M)	2	24,90	12,451	0,071 <sup>ns</sup>	3,26	5,25
Interaction W x K	6	7401,65	1233,609	7,05**	2,36	3,35
Interaction W x M	4	36,71	9,177	0,052 <sup>ns</sup>	2,63	3,89
Interaction K x M	6	239,50	39,916	0,228 <sup>ns</sup>	2,36	3,35
Interaction W x K x M	12	165,01	13,751	0,079 <sup>ns</sup>	2,03	2,72
Galat	36	6299,63	174,99			
Total	72	116131,77				

Description = df: degrees of freedom, NS: Number of Squares, CS: Central

\*\* : Significant difference in the degree of confidence of 95% and 99%  
 ns: not significantly different

**Tabel 2.** Influence Interaction of various immersion time with different concentration *L. acidophilus* for total *E. coli* in Swordfish Stew (*Auxis rochei*).



Immersion	Concentration <i>L. acidophilus</i>			
	k (Control <i>E.coli</i> )	k <sub>1</sub> (10 <sup>6</sup> cfu/ml)	k <sub>2</sub> (10 <sup>8</sup> cfu/ml)	k <sub>3</sub> (10 <sup>11</sup> cfu/ml)
w <sub>1</sub> (30 mnts)	275 c	36,48 b	8,3 ab	0.36 a
w <sub>2</sub> (60 mnts)	442,5 c	26,26 b	6,92 ab	0.36 a
w <sub>3</sub> (90 mnts)	617,5 c	36,03 b	4,8 ab	0 a

Description: The letter with the same notation in all directions showed no significant difference in the degree of confidence of 95% according to Duncan test  
 = Inhibition of growth The biggest *E.coli*

Duncan test results in Table 2 shows that the number of *E. coli* significant than all treatment groups. All soaking time 30 minutes, 60 minutes and 90 minutes to give effect to the amount of growth of *E. coli* that is an average decrease in the number of *E. coli* along with the increasing immersion time given.

During immersion 30 minutes, 60 minutes and 90 minutes of competition between *L. acidophilus* with *E. coli* to obtain nutrients. During the competition that may occur beginning with the decline in food supply, increased density, and production of the metabolites (26). Interactions between the concentration of *L. acidophilus* 10<sup>11</sup> cfu / ml with dipping time 30 minutes, 60 minutes and 90 minutes caused the density of *L. acidophilus*, because the generation time of *L. acidophilus*, which is faster than *E. coli*. Density of *L. acidophilus* is one of the factors that influence the effectiveness of *L. acidophilus* in suppressing the growth of *E. coli*, due to increased density can affect the competition of nutrients (15).

Based on Table 2 also shows that the difference in decline - average amount of growth of *E. coli* on each - each concentration *L. acidophilus*. In the treatment of 10<sup>11</sup>cfu/ml *L. acidophilus* concentration (k<sub>1</sub>) after 30 minutes soaking time and 60 minutes (w<sub>1</sub> and w<sub>2</sub>) showed decrease in the number of *E. coli* is the same ie 0.36 cells / g, whereas immersion time of 90 minutes showed highly significant differences in the number of *E. coli* was 0 cells / g compared with the concentration of *L. acidophilus* as 10<sup>8</sup>cfu/ml (k<sub>2</sub>) and 10<sup>6</sup>cfu/ml (k<sub>1</sub>) and control (k). Based on the results showed a decline in the number of

bacteria *E. coli* along with the increasing concentration of *L. acidophilus* and the length of time of immersion. The recommended dose of probiotics is  $10^7$  to  $10^9$  so that when given less than that then the process will not be achieved so that a balance can not be referred to as probiotics (27).

Therefore, to inhibit the growth of *E. coli* in the tuna meat stew is required concentration *L. acidophilus*  $10^{11}$  cfu/ml so that the growth of *E. coli* to be absent. This condition proves that the addition of various concentrations of *L. acidophilus* and various immersion time has a different effect on the growth of *E. coli*.

Results Analysis of Variance in Table 1 shows that various methods of inoculum *L. acidophilus* preventively (m1), curative (m2) and simultaneous (m3) does not give real effect on the number of *E. coli*, therefore it is not followed by Duncan test. This shows that all methods of inoculum *L. acidophilus* did not indicate any difference in suppressing the growth of *E. coli* in stew meat tuna because it is only affected by the length of time of immersion and the concentration of *L. acidophilus* given. *L. acidophilus* active biochemical processes that produce antimicrobial compounds such as lactic acid, bacteriocin and hydrogen peroxide so as to inhibit the growth of *E. coli* (28).

Mechanism of inhibition of *L. acidophilus* against *E. coli* is by destabilization of the cytoplasmic membrane. *L. acidophilus* is a lactic acid bacteria that are homofermentatif by using substrates that are available on the environment by the end of the primary metabolites of lactic acid. The presence of lactic acid as a product of metabolism can be as one factor inhibiting the growth of *E. coli* because it causes acid atmosphere (Betty, 1999 in 29).

## CONCLUSION

*L. acidophilus* can inhibit bacterial growth of *E. coli* by giving *L. acidophilus* concentration of  $10^{11}$  cfu / ml is able to reduce the number of *E. coli*  $10^8$  cfu / ml to 0 cells / g after 90 minutes soaking time. The greater the concentration of *L. acidophilus* provided and the longer the immersion time influence on the growth of *E. coli*. Various methods of inoculum *L. acidophilus* in preventive, curative and simultaneously also can reduce the number of bacterial contaminants of *E. coli*.

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