

The Examination of Lactate Acid Bacteria *Streptococcus thermophilus* AST 6 Ability from Fish Digestion Tract to the Different Temperature

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Abstract- This research was carried out to determine the ability of lactate acid bacterium (LAB) isolated from fish digestion tract to the some range of temperatures 12, 25, 40 dan 45. Moreover, it is to determine the AST 6 LAB growth isolated from fish digestion. Further, this research is beneficial as information for the livestock industries to the use of LAB isolates and is expected to be useful in LAB growth study for stuents, food processing industries, and animal husbandry.

The method used was isolating LAB *Streptococcus thermophilus* isolates from fish waste in their fish digestion. Then, LAB isolates incubated in liquid media using MRS broth (5 ml) for 24 hours in difference temperature: 12, 25, 40, and 45. Positive reaction of growth character LAB isolates measured the number of optical density (OD) in 620 nm wavelength with spectrophotometer (Spectronic 21; Milton Roy Company, USA).

AST6 LAB isolation result are mesophilic bacteria, they live in non-ruminant digestion tract. In line with Peterson (1992) opinion, he states that mesophilic bacteria live in non-ruminant animal, they grow at optimum temperature 20 – 40°C, minimum growth (10 – 20°C), and maximum growth (40 – 45°C). The conclusion is LAB isolates grows well at optimum temperature 20 – 40°C, minimum growth (10 – 20°C), and maximum growth (40 – 45°C).

Index Terms- Isolate, Lactic Acid Bacteria, dan Streptococcus thermophilus

I. INTRODUCTION

Microflora has important role for digestion tract and the health (Soeyenbos, 1987; Wren, 1987 dalam Soetanto *et al.*, 2001). The balance between beneficial and pathogenic microbial is the important factor in determining product as well and animal health. The type of bacteria *Lactobacilli* and *Streptococci* are microflora having a role in the balance (Soetanto, 2001). Probiotic term is introduced by Lily dan Stilwell (1965), it is intended as spur growth factor by microorganism. Probiotic comes from Greek which means improving live (*pro life*). Parker (1974) uses that term for “organism and substance” which benefits for animal by improving microflora balance. In addition, Fukker (1989) defines probiotic as feed supplement which contains microbial life to improve the balance of digestive tract microbial. Nowadays, probiotic is life microbial consumption as a food additive for health.

Ecologically, Lactic Acid Bacteria can be isolated from any habitats including human, animal, plant, milk product, meat

product, hay, vegetables, and beverages. Since temperature has close relation to the enzyme synthesize, it has an effect for the growth, reproduction, and microorganism survival. LAB is only able to grow at temperature 20°C-40°C (Stamer, 1979). The examination of temperature range of LAB growth is needed, whether the difference range temperature they capable to grow and used as one of probiotic parameter. The requirements of a microorganism can be used as probiotic are:

- Tolerance to the acidity (low pH) and bile.
- Produce lactic acid and anti microbial compounds.
- Able to attach in intestinal wall.
- Resistent to some different environment temperatures especially in digestive tract and processing temperature.

Based on the description above, it can be seen that lactate acid bacterium has temperature range in order to grow optimally. The temperature range of each LAB type is difference, so the research dealing with optimum temperature for growth of each LAB isolated from fish digestion is needed.

Formulation of the Problem

How does the growth of LAB isolated from fish digestive tract to some temperature range 12, 25, 40, and 45?

The Objectives

To determine the growth of LAB isolated from fish digestive tract to some temperature range 12, 25, 40, and 45.

The Significance

1. This research is the basic study which is expected can be used as information source for husbandry industries to the use of LAB isolates.
2. This research is expected to be useful in study relates to the LAB growth for students, food processing industries, and animal husbandry.

II. RESEARCH METHOD

Materials

Mikrobial sources

LAB isolated from fish intestinal tract waste.

Chemical materials

Chemical materials used are MRS broth (Oxoid and Pronadisa brand), gelatin, glycerol 20%, lactose, glucose, lactic acid 90%, aquadesh, NaOH dan HCl to adjust the pH, alcohol 70%, generating kit CO₂, methylated spirits, determination lactic

acid level reagent, *gram staining* reagent, H₂O₂, CO₂, and bile salt (Oxoid brand).

A. Tools

1. Tools

- | | | |
|---------------------------|-----------------|---------------------|
| a) Petridist | j) Erlenmeyer | s) Thermometer |
| b) Beaker glass | k) Microscope | t) Paper labels |
| c) Measuring cup | l) Object glass | u) Cotton |
| d) Measuring pipette | m) Ose needle | v) Rubber |
| e) Test tube | n) Stove | w) Oven drive |
| f) Autoclave | o) Micropipette | x) Spectrometer |
| g) Test tube rack | p) Bunsen | y) Cuvet tube |
| h) LAF (Laminar Air Flow) | q) Loupe | z) Centrifudge tube |
| i) Analytical scales | r) pH meter | |

B. Research method

MRS MEDIUM

MRS broth (Oxoid) composition:

- | | | |
|---|--|--|
| a. 1% peptone | e. 0,1% tween 80 | i. 0,02% MgSO ₄ .7H ₂ O |
| b. 0,8% lab lemco powder | f. 0,2% dipotasium hydrogen phosphate | j. 0,005% MnSO ₄ .4H ₂ O |
| a. 0,4% yeast extract | g. 0,5% sodium asetat. 3H ₂ O | |
| b. Subtrate (glucose with concentrate based on treatments 0.5%, 1%, 1.5%, 2%) | h. 0,2% citric triamonium | |

MRS broth (Pronadisa) composition:

- | | | |
|-------------------------------|---|----------------------------|
| a. 1% bacteriological peptone | e. 0,2% dipotasium hydrogen phosphate | i. 0,02% Magnesium sulfate |
| b. 0,8% meat extract | f. 0,1% tween 80 | j. 0,005% Mangan |
| c. 0,4% yeast extract | g. 0,5% sodium asetat.3H ₂ O | |
| d. 2% dextrin | h. 0,2% citric ammonium | |

Materials:

Lactic Acid Bacteria isolated (LAB, MRS broth, TCA 10%, CuSO₄ 20 %, distilled water, Ca(OH)₂, ice water, para hidroksibipenil solution, concentrated H₂SO₄, alcohol 70%)

Research Variables:

Independent Variables : temperature 12 , 25 , 40, and 45

Dependent Variables : the bacteria growth which measured OD (Optical Density)

Procedures

a. Preparation

- The tools used were sterilized by autoclaving at temperature 121°C with a pressure 1 atm for 15 minutes.

- MRS broth media: →

MRS Liquid Medium:

1. Dissolve 5, 2 gram MRS into 100 ml aquadesh, diluted with strirer until homogoneous.
2. Ensure medium pH 6, 5.
3. Heat medium until boiling in 3 times then divide 4 ml for each tube.

4. Digasi with CO₂ till anaerobic condition then closed and sterilized by by autoclaving at temperature 121°C with a pressure 1 atm for 15 minutes.

- b. Activate LAB isolated from fish digestive tract. Inoculating the culture result as much as 10% in liquid MRS media (MRS broth) 2 x 10 ml. Then, incubate that culture at temperature 37°C for 24 hours.

c. Growth examination to the temperature;

LAB isolates were incubated in liquid media using MRS broth (5 ml) for 24 hours at different temperature: 12, 25, 40, and 45. Positive reaction of growth character LAB isolates was measured the number of optical density (OD) in 620 nm wavelength with spectrophotometer (Spectronic 21; Milton Roy Company, USA).

III. RESULTS AND DISCUSSION

Isolates AST 6 survival test at refrigerator temperature (12°C), room temperature (25 °C), 40°C dan 45°C, as shown in the Table 1.

Table. 1. Survival LAB AST6 to the Temperature

	JAM KE-										
TEMP.	0	0,5	1	2	3	4	5	6	7	8	9
12°C	0,192	0,187	0,194	0,204	0,196	0,203	0,209	0,217	0,220	0,226	0,227
20oC	0,190	0,198	0,210	0,245	0,315	0,405	0,514	0,627	0,717	0,799	0,864
40oC	0,190	0,220	0,244	0,406	0,664	0,852	0,927	0,990	1,019	1,025	1,031
45oC	1,920	0,229	0,243	0,380	0,582	0,787	0,847	0,898	0,935	0,944	0,948

	JAM KE-									
SUHU	10	12	14	16	18	20	21	23	24	
12°C	0,230	0,253	0,247	0,255	0,282	0,273	0,276	0,298	0,307	
20°C	0,906	1,004	1,050	1,084	1,090	1,093	1,100	1,109	1,099	
40°C	1,028	1,041	1,064	1,069	1,090	1,094	1,107	1,118	1,113	
45°C	0,951	0,961	0,993	1,010	1,024	1,031	1,049	1,067	1,057	

Figure 1: LAB AST6 Endurance Test to Temperature

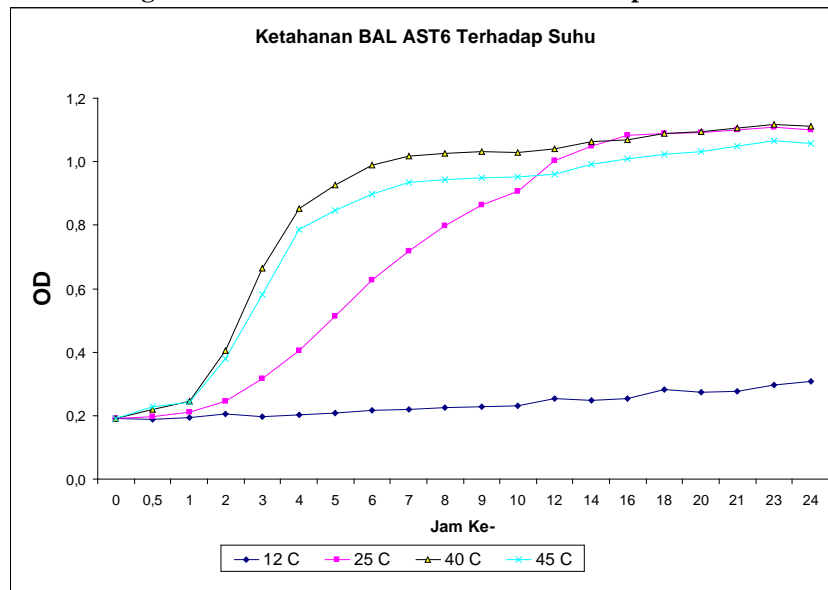
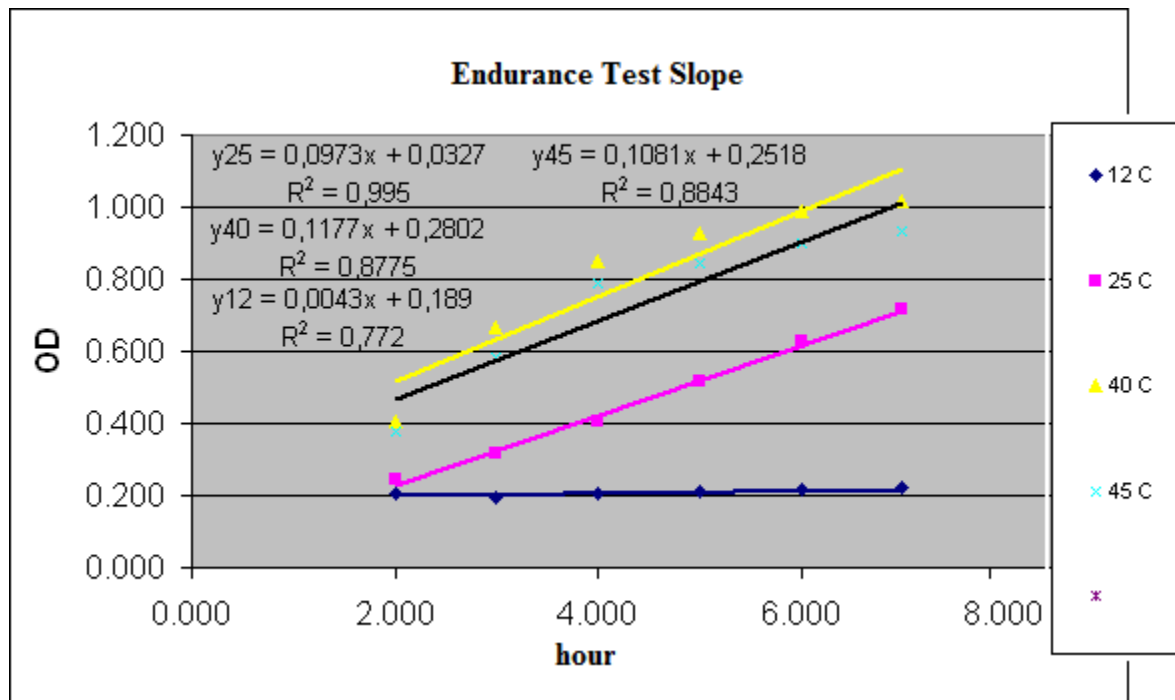


Figure 2: Growth Slope to Different Temperature



AST6 LAB isolation result are mesophilic bacteria, they live in non-ruminant digestion tract. In line with Peterson (1992) opinion, he states that mesophilic bacteria live in non-ruminant animal, they grow at optimum temperature 20 – 40°C, minimum growth (10 – 20°C), and maximum growth (40 – 45°C). By knowing the growth temperature range will facilitate the further application.

Ecologically, Lactic Acid Bacteria can be isolated from any habitats including human, animal, plant, milk product, meat product, hay, vegetables, and beverages. Although these microorganism exist in various places but a few species adapts specific environment, such as, is only able to grow on the salt levels, sugar, higher alcohol, 20 – 40 °C temperature, and is able to ferment various types of mono and disaccharide. Some species are capable to form antagonistic compounds to LAB whicg dominate their environment. (Axelsson, 1998).

IV. CONCLUSION

The result of this research are LAB from fish digestive tract are able to survive at temperature 45 °C and AST 6 LAB can be probiotic.

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