Isolation and characterization of Lactic Acid Bacteria 
(*Lactobacillus sp*) from strawberry (*Fragaria vesca*)

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Abstract. The principle of making yoghurt is fermentation of milk with the addition of lactobacillus (LAB) and Streptococcus bacteria. LAB often found naturally in fruits and vegetables. One of the fruits that is thought to contain lactic acid bacteria is strawberry (*Fragaria vesca*). Strawberry are thought to be one of the natural media of bacteria including lactic acid, because strawberry contain various chemical compositions that are needed as a substrate for LAB. The purpose of this study is isolate LAB from strawberry and determine the type of LAB produced microscopically. Research methods, the ingredients used are strawberry obtained from community plantations in Aie Angek, Tanah Datar district, with Merck NA media, oxoid MRSa, 0,9% NaCl, crystal violet paint from biological laboratories of Universitas Negeri Padang. Isolation LAB from strawberry done in two ways, the first with plant strawberry tissue directly into the NA medium and the second with fermentation strawberry and then plant the strawberry into the MRSa medium with streak plate methods. The isolates obtained were identified microscopically using a microscope with gram staining method. From the research that has been done, the following result are obtained: (1) Plant strawberry tissue directly into NA medium and gram staining, there were 2 colonies of gram positive bacteria with bacil cell form. (2). Fermentation strawberry from the results, there were 2 colonies gram positive bacteria with bacil cell form. We can identify this colonies as *Lactobacillus bulgaricus*.

1. Introduction

The principle of making yoghurt is fermentation of milk with the addition of *Lactobacillus* (LAB) and *Streptococcus* bacteria. With this fermentation, the taste of yogurt will become acidic, because there is a change in lactose to lactic acid by these bacteria (Fevria, R, 2016).

In addition to fermented products milk lactic acid bacteria are often found in various agricultural products. Some sources explained that fruits and vegetables such as wheat, rice, cassava (Reddy, 2008), soybean waste (Malik, 2008) fruit and vegetable pickles (Kusumawati, 2003), drinks and fruit (Plessis, 2004), papaya, salak (Kurniawan, 2005) and durian, pineapple, cacao, banana (Nurhayati, 2011).

Currently, some lactic acid bacteria that have been isolated from fermented foods include cayenne pepper (Rustan, 2013), pickled mustard greens (Rachmawati, 2005), yellow passion fruit (Sari, 2013) and purple passion fruit (Fatimatuz zahro, 2014) as bacterial inhibitors pathogen. Whereas mango, tomato, cabbage, lettuce, long bean are potential sources of lactic acid bacteria (Noordiana, 2013).
Based on the above background and the possibility of the presence of lactic acid bacteria found in strawberries because of the large benefits of lactic acid bacteria, a study will be conducted "Isolation of Lactic Acid Bacteria (Lactobacillus sp) (LAB) from Strawberry (Fragaria vesca).

To make it easier to get a pure starter in making yoghurt, it is necessary to explore the source of LAB from natural ingredients such as strawberry. Formulation of the problem is: How many LAB live on strawberry and What types of LAB are found on strawberry. The purpose of this study is: Isolate LAB from strawberry and Determine the type of LAB produced microscopically.

2. Research Methods

2.1 Time and Place
This research was conducted in the biology laboratory Universitas Negeri Padang in August – September 2018.

2.2. Procedure
The ingredients used are strawberry obtained from community plantations in Aie Angek, Tanah Datar district, with Merck NA media. Oxoid MRSa, 0,9% NaCl, 3% H2O2 solution, crystal violet paint, 96% alcohol and safranin obtained from biological laboratories UNP.

2.3. Equipment
The tools used are jars, cutter knives, analytic scales, measuring flasks, erlenmeyer flasks, petri dishes, incubators, autoclaves, object glass, hot plate, bunsen twizeers, porcelain cups, metal spaters, scratch gears, tube racks, microscopes.

2.4. Implementation of Research
2.4.1. Sample Preparation
Strawberry samples were taken from the community plantations in Aie Angek, Tanah Datar district with perfect maturity and red. After preparing the sample, it was continued by making NA medium, sterilizing medium, pouring medium and checking medium. Make triopchlorite 1% and 2%.

2.4.2. Planting Samples
Planting samples can be done in two ways, namely: (1) Direct tissue planting can be done by means of sterilizing the surface of the sample with a triopchlorite solution, after sterilizing plant the sample tissue into the NA medium. Then do the observation for 1 x 24 hours and 2 x 24 hours. After growing the bacteria do purification of bacteria. After purification, observe the morphology of bacterial colonies. (2). Fermentation, can be done by way of sterilizing the surface of the sample which is cut aseptically, then put into a jar that has been sterilized and covered with aluminum foil and incubated for 5 days. After that, it immediately breaks the MRS medium using an ose needle. After the incubation process is complete, it is continued by isolating the colonies that grow respectively based on the morphological differences of the bacterial colonies in the petri dish every quadrant. Isolation was carried out until isolates or single colonies were obtained from each petri dish.

2.4.3. Identification of Lactic Acid Bacteria (LAB)
The obtained isolates were identified by the macroscopic method, by looking directly at the morphology of bacterial isolates that grow on the medium including the shape and color of the bacteria. And microscopic method using a microscope with gram coloring method. Gram staining is done with applying bacteria over the glass object, lubricating it then dropping paint gram A, let it sit for 1 minute, dropping paint gram B, let it sit for 1 minute, drop paint gram C, let it sit for 30 seconds, drop paint gram D, let stand 2 minutes. Then covered with a glass cover and pressed with emersion oil then observed the shape and color of the cell.

3. Results and Discussions
3.1. Isolation Lactic Acid Bacteria (LAB)
Strawberry in ancient Greece was appointed as a symbol of the Goddess of Love is able to prevent coronary heart and suppress high blood pressure. Strawberry can also overcome digestive problems,
liver, rheumatism, arthritis and gout. The chemical content of strawberries is very large and contains sufficient nutrition including protein, fat, carbohydrates, calcium, phosphate, iron, energy, water and vitamins.

Lactic Acid Bacteria is one of the organisms that ferment food through fermentation of carbohydrates and generally produces large amounts of lactic acid. These bacteria contribute significantly to the improvement of flavor, texture and shelf life of fermented products. The use of BAL is because lactic acid bacteria is called a food grade microorganism which is a microbe that is not at risk to health because it does not produce harmful toxins in food but has the opposite function that is good for health.

The results of isolation carried out by planting strawberry tissue directly into the NA medium obtained two colonies called isolates as many as two isolates. Can be seen in Figure 1.

| Isolate code | Picture of the Colony | Gram Coloring +/- |
|--------------|-----------------------|-------------------|
| JS 1         | ![Picture of JS 1 Colony](image1) | Gram: Positive Cell Shape: Basil |
| JS 2         | ![Picture of JS 2 Colony](image2) | Gram: Positive Cell Shape: Basil |

**Figure 1.** Isolation of Laktobacillus Bacteria from Strawberry Fruit Using Medium NA (Nutrient Agar)

Table 1. Advanced Test Using Selective Medium (MRSA)

| Isolate code | Selective Medium MRSA | Information |
|--------------|------------------------|-------------|
| JS 1         | -                      | Not *Lactobacillus* |
| JS 2         | -                      | Not *Lactobacillus* |

After further testing using selective media (MRSA), the results are as follows, as in table 1. Andrianto (2008), said that in life Lactobacillus bulgaricus bacteria have several benefits including increasing milk digestibility, stimulating interferon production and tumor necrosis factor, regulating the immune system, helping lipid metabolism, controlling cholesterol levels, producing antibiotics (antibiotics) natural, and can inhibit the proliferation of unwanted microorganisms.

### 3.2. Strawberry Fermentation

The results of isolation were carried out by fermenting strawberries for five days and then breaking down into MRS specific medium to obtain two colonies which were called isolates as much as two isolates, as in figure 2.
Lactobacillus bulgaricus is a gram-positive, rod-shaped, sometimes paired and does not form endospores. In milk, Lactobacillus bulgaricus will convert lactose to lactic acid. This bacterium is homofermentative, with the optimum temperature for growth of around 45°C. The optimum condition for growth is a little acid around pH 3.5.

### 3.3. Identification of Isolate Isolation Results
Bacterial identification aims to determine the specific characteristics possessed by the isolates obtained which have the same characteristics as the desired bacteria. In this study identification of isolates was carried out microscopically using a microscope to see the shape of bacterial cells. Microscopic identification of bacteria was carried out by gram staining. Morphology of LAB results from gram staining can be seen in Figure 1 and Figure 2.

Observations on microscopy with gram staining showed the results of testing that the yellowish-colored bacterial cells and basil cell forms in Figure 1 and milky white with Bacillus cell form in Figure 2, showed that lactic acid bacteria (LAB) isolated belong to the class of gram-positive bacteria allegedly from the genus Lactobacillus for figure 2, while image 1 is not classified as Lactobacillus. Gram-positive bacteria have a characteristic cell wall with thicker peptidoglycans so that the absorption of color from paint Violet crystals absorbed in the cell will survive even though washing is done using laxative paint (alcohol-lugol solution) which is expected to be able to dissolve the first color paint.

![Image](image1.png)

**Figure 1.** Strawberry Fermentation

![Image](image2.png)

**Figure 2.** Strawberry Fermentation

### 4. Conclusion
From the research that has been carried out it can be concluded that there were 2 lactic acid bacteria colonies from LAB isolation from strawberry by planting tissue directly into NA media but not classified as Lactobacillus and by fermenting and planting it into MRSa media that there were 2 lactic acid bacteria which is classified as Lactobacillus. LAB obtained is lactobacillus because of the results of gram staining is gram + bacteria with basil cell form.

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