The role of lactic acid bacteria (*Lactobacillus sp yel133*) from beef in inhibiting of microbial contaminants on various fillers of starter culture

Yunilas and E Mirwandhono

Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Indonesia
E-mail: yunilas11@yahoo.co.id

Abstract. The role of Lactic Acid Bacteria (LAB) on the starter culture can be seen from the ability to grow and suppress the growth of microbial contaminants (fungi). The research aimed to investigate the role of LAB (*Lactobacillus sp YEL133*) in inhibiting microbial contaminants (fungi) on starter cultures of various fillers. The materials used in this research was *Lactobacillus sp YEL133* from beef and various fillers (rice flour, corn starch and wheat flour). The research methods used completely randomized design (CRD) with 3 treatments and 4 replications. The treatments of this research was P1 (rice flour), P2 (corn starch) and P3 (wheat flour) that inoculated with *Lactobacillus sp YEL133*. Parameters which is observed such as: growth of lactic acid bacteria, total microbes and total fungi as microbial contaminants. The results showed that starter culture with a filler material of rice flour produce lactic acid bacteria and microbes were highly significant (P <0.01) for corn starch and wheat flour, as well as able to suppress the growth of microbial contaminants (fungi). The conclusion of the research is the use *Lactobacillus sp YEL133* can suppress the growth of fungi on the starter culture using rice flour.

1. Introduction
Lactic acid bacteria that is use as starter cultures should be able to meet the criteria: a) be able to compete with other microorganisms, b) quickly produce lactic acid, c) be able to grow at a salt concentration of less than 6%, d) capable to react with a concentration of less than 100 mg / kg, e) capable to grow at temperatures between 15-40°C, f) include homofermentatif bacteria, g) does not generate peroxide in large quantities, h) can reduce nitrite and nitrate, i) can improve the flavor of the final product, j) does not produce amino acid compounds, k) can kill spoilage bacteria and pathogens, and l) synergistic with other starter compounds [1]. LAB (lactic acid bacteria) was able to convert sugars (including lactose) and other carbohydrates into lactic acid. These not only provides a unique sour taste of fermented dairy food such as fermented milk, but also acts as a provider by reducing the pH and create opportunities for harmful organisms to grow a little more.

Starter culture is a strain of microorganisms’ that has been selected and is known to carry out metabolic activities that can improve the characteristics of the fermented material. Furthermore, starter culture is a selected strain of food-grade microorganism that is known to have metabolic activity and other stable property that is used in producing fermentation products with desired or attributes characteristics [2]. Starter functions include forming flavor (which is a product of organic acids, carbonyl component and partial hydrolysis of proteins and fats); inhibit unwanted microorganisms;
improve the texture of fermented products such as viscosity and cohesiveness also contribute as a functional food product with the use or addition of probiotic microorganisms [3], [4].

Pickling starter is generally done by drying [5], to maintain the viability and the particular traits of bacteria such as the acid-forming activity, produce aroma, texture and shape as the nature of the probiotics [4]. Heat stress that occurs during the process of drying and dry storage is the major cause of the loss of bacteria activity [6], also the stress of acid [7], nutritional deficiencies [8], osmotic stress and oxidative stress [9] affect to the performance of the starter.

The use of bacteriocin-producing bacterial strains as starter cultures or protective culture recently developed and is able to control the presence of pathogens and spoilage bacteria in ready food products [10]. Culture that is often used for the fermentation of meat and commercially available come from the Streptococcus [11], class Micrococcus [1], Lactobacillus plantarum, Lactobacillus sake, L. Curvatus, Pediococcus inacidaictici and the right combination with P. Pentosaceus [12]. Naturally, there are species of lactic acid bacteria that grow on pure beef, one of which is L. plantarum [13].

Starter culture in the manufacture of sausages can be divided into two categories. The first generation contains lactic acid bacteria derived from plant material. The second generation comes from meat material that is specifically adapted to the ecology of fermented meat [14]. Starter cultures of lactic acid bacteria isolated from other than meat still less adaptive and less than optimal for the meat fermentation is characterized by fluctuating viability during the process [15] and [16].

Commercial dry cultures for fermented sausages have a total lactic acid bacteria at least $10^6$ CFU / g. Lactic acid bacteria Lactobacillus sp YEL133 is one of lactic acid bacteria isolated from beef can be used as starter cultures in the making probiotic sausage [17]. However, the bacteria Lactobacillus sp YEL133 need to be tested role as starter cultures in inhibiting the growth of fungi, testing the viability on the various fillers.

2. Material and Methods

2.1. Materials and Equipment

Probiotic sausages dry starter culture is made from pure cultures of lactic acid bacteria (Lactobacillus sp YEL133) isolated from beef purely based on research results of [17]. The medium used is MRSB (de Mann Rogose Sharpe Broth) and MRSA (de Mann Rogose Sharpe Agar) brand Merck, NB (Nutrient Broth), NA (Nutrient Agar), peptone for casein, yeast extract, CaCO3, buffered peptone water (BPW), ethanol, acetone, glycerol, grandula agar, 70% alcohol, 96% alcohol, 99% acetone, BCP (brome creasol purple), H2O2, violet crystal, lugol, acetone, safranin, cotton, distilled, spirits, vortex, hot plate, autoclave, incubator, vacuum cabinets, wire loop, laminar air flow, micropipette, analytical balance, oven, Bunsen burners, petridish, pH meter, a spatula, a 1.5 mL tube, vortex and glassware.

2.2. Methods

2.2.1. Purification: Purification is done by growing lactic acid bacteria isolates from the results of previous research on medium de man Rogose Sharpe broth (MRSB) and de man Rogose Sharpe agar (MRSA) alternatively to seek a uniform pure colony. Purity test then followed by biochemical tests based on its ability to ferment several types of sugars to ensure that the culture used is a pure culture. Testing is done by fermenting culture on sugar medium.

2.2.2. Refresher: Refresher is done by growing isolates in MRSB media for 24 hours at a temperature of 37°C. Refresher is continue until the culture could adapt to medium that is characterized by clouding on the growth media. Refresher aims is to update and expand the culture of lactic acid bacteria before it is used as a starter culture of fermented sausage.

2.2.3. Breeding: Culture that has been freshened up, then inoculated in a medium containing 2% skim milk, which aims to reproduce and adapt to the culture medium. Medium then incubated at a
temperature of 37°C for 48 hours. The result is called the main culture. This process is continued until it obtained the between culture and work culture.

2.2.4. Manufacture Dry Culture and Viability Test: Culture that has been bred will then added with fillers respectively 50% rice flour, corn flour and wheat flour. The aim of using filler material in the manufacture of dry culture is to preserve the activity of starter cultures during drying. Starter that has been added fillers will then dried using chemo reaction method with water content of culture almost ±10%.

2.2.5. Methods: The research methods used completely randomized design (CRD) with 3 treatments and 4 replications:

P1 = rice flour
P2 = corn starch
P3 = wheat flour

2.2.6. Parameter: Parameters measured include: the viability of lactic acid bacteria, total microbial and total molds (fungi) as microbial contaminants.

3. Results and Discussion

3.1. Total Lactic Acid Bacteria (LAB) Dry Starter Culture Sausages

The experimental results obtained total lactic acid bacteria (LAB) on the dry starter cultures varies with a range of $1.10 \times 10^6$ CFU / g (P2) - $1.70 \times 10^7$ CFU / g (P1) Table 1. The analysis showed that the treatment of various fillers was highly significant (P <0.01) to total lactic acid bacterial starter culture dried sausage.

| Filler material | $\Sigma$ LAB (colony/g) |
|-----------------|------------------------|
| Rice flour      | $1.70 \times 10^7$ A   |
| Corn flour      | $1.10 \times 10^6$ C   |
| Wheat flour     | $1.40 \times 10^7$ B   |

Table 1. Total Lab In The Dry Starter Culture Of Sausages

Description: different super scripts letters in the same column indicate significantly different (P <0.01)

Duncan advanced test showed that the total LAB dry starter culture with rice flour filler treatment were significantly (P <0.01) higher than other treatments. This is because the starch content of rice flour was higher than corn and wheat flour. High starch content will increase the water holding capacity in the medium or cells, so it can protect microbial cells during the drying process. Consist to a statement from Chaplin [18] that one of the function of starch is a binder to water (water binder).

[19] states that the content of starch in wheat flour is about 70% while the starch content of rice flour is 90% of the dry weight. According [11], the use of heating medium that contains carbohydrates will increase the heat resistance of microbes contained in there. Components of the initial medium in microbes before being dried are mostly water. Rice flour has a higher starch content, so the amount of water and microbes that are bound and protected from direct exposure to heat during the drying starter using rice flour filler material will be greater.

Besides the starch content of rice flour, the properties of starch and protein in rice flour can withstand and protect Lactobacillus sp YEL133 isolate to heat during the drying process, resulting in microbial viability higher than other treatments.

Differences in total LAB dry starter culture is also influenced by the levels of amylose and amylopectin starch constituent. Amylose is easily bound to water; this is because amylose consists of hydroxyl chain in large numbers so it could increase the water holding capacity in the material. Amylose prepare amorphous region and amylopectin prepare crystalline regions of starch granules. Amorphous region will absorb water earlier than crystalline regions because amylose is more
hydrophilic. Amylose is hygroscopic; it can increase water absorption and gel formation easier because the straight chain could easily form a three-dimensional network. According to [20], amylose prepares amorphous regions and amilopectin prepare crystalline regions of starch granules. At the time of gelatinization, amorphous region absorbs water earlier because amylose is more hydrophilic, but amylose undergoes syneresis and crystallized faster.

Rice flour has amilopectin levels higher than corn and wheat flour so that the amount of water that is bound also greater after amylose. Therefore, the dry starter culture with higher levels amilopectin excipients capable of producing higher cell viability that is shown by the total LAB generated is higher in rice flour rather than corn and wheat flour.

3.2. Total Fungi Dry Starter Culture Sausage

Mean total fungi dry starter culture sausage on various types of fillers can be seen in Table 2. The results show the average total fungi in dry starter cultures sausages ranging from $1.46 \times 10^4$ to $3.00 \times 10^4$ CFU/g, Table 2.

Total fungi obtained allegedly came from contamination that occurred during the manufacturing process of dry starter, especially in the drying process and also influenced by the type of flour used as filler.

Results of analysis of variance showed that the treatment of various fillers is highly significant ($P < 0.01$) of the total fungi dry starter cultures sausage. Duncan advanced test showed that total fungi in dry starter culture made from wheat flour is not significantly different from rice flour, but highly significant with cornstarch. Allegedly due to the content of gluten in wheat flour is higher than corn flour. The interaction between water and wheat flour will produce gluten that has strong dough structure. This is thought to inhibit the penetration of fungi into the starter.

| Filler Material | $\Sigma$ fungi (colony/g) |
|-----------------|---------------------------|
| Rice flour      | $1.0 \times 10^4$         |
| Corn flour      | $3.00 \times 10^4$        |
| Wheat flour     | $1.46 \times 10^4$        |

Description: different super scripts letters in the same column indicate significantly different ($P < 0.01$)

Total fungi were lower in the treatment of dry starter culture in rice flour and wheat flour than corn flour. It is affected because the total LAB that is obtained is higher in that treatment. The high total LAB will produce a high lactic acid, causing the pH of the medium is low. Low pH medium will suppress the growth of pathogenic microbes, including fungi as a contaminant. LAB also produces other compounds, namely hydrogen peroxide, diacetyl, carbon dioxide, reuterin and bacteriocins which also serves as an antimicrobial.

3.3. Total Microbial Dry Starter Culture Sausages

Total microbial in dry starter culture is the total number of microbes contained in dry starter culture. The mean total microbial in dry starter culture sausages ranged between $5.65 \times 10^5$ (CFU/g) - $8.00 \times 10^6$ (CFU/g), Table 3.

Based on the analysis of variance, the treatments of various types of fillers have a very significant effect ($P < 0.01$) of the total microbial dry starter culture sausage. Duncan advanced test, indicate that the interaction of treatment using rice flour as a filler material, produces a number of microbes that is significantly higher than other treatments. This is because the total microbial dry starter culture is influenced by the total LAB and also influenced by the total fungi as microbial contaminants in the dry starter culture.
The high total microbes on the treated filler rice flour is due to the high total lactic acid bacteria that being produced. Total high lactic acid bacteria are determined by high starch content. This is consistent with the statement from [18] that one of the functions of starch is as a water binder. According to [11], rice flour has a higher starch content, so the amount of water and microbes that bound and protected from direct exposure to heat during the drying starter using rice flour filler material will be even greater.

While the total microbial in treatment using corn flour is lower than other treatment. This is because the starch content of corn was lower than rice and wheat flour, so that the water-binding power is lower. Thus the ability to protect the microbes from direct exposure to heat during the drying is also low. This leads the viability power of microbes also low especially LAB.

4. Conclusions
Based on the results of this study concluded that Lactobacillus sp YEL133 can suppress the growth of fungus in dry starter culture using rice flour.

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Table 3. Total microbes in the dry starter culture sausages

| Type of filler material | Σ Live microbes (colony/g) |
|------------------------|--------------------------|
| Rice flour             | 8.00 x 10^6 A            |
| Corn flour             | 5.65 x 10^5 C            |
| Wheat flour            | 7.00 x 10^6 B            |

Description: different super scripts letters in the same column indicate significantly different (P <0.01)
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