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Activity of Inhibition Bacterial Pathogens from Symbiotic Yogurt of Yellow Yam Tuber with the Addition of Probiotics strains *L. rhamnosus* and *Bifidobacterium ssp*

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ABSTRACT

The advantages of yogurt products compared to others functional food is the inhibitory activity of pathogenic bacteria. The combination of bacterial culture in yogurt continues to be developed thus gaining a bacterial culture that can maximize health effects especially to prevent the growth of pathogenic bacteria (normal microflora) in the gastrointestinal tract. The purpose of this study to see the influence of the addition *L. rhamnosus* and *Bifidobacterium ssp* probiotics against the ability of sinbiotics yogurt utilizes the yellow yam tuber in inhibiting pathogenic bacteria.

The study uses complete random design with 3 different treatment types including 1 control and 3 repetitions. Inhibition of pathogenic bacteria by simbiotic yogurt is influenced by the combination of bacteria used. Moreover, the efforts to maximize health effects when consuming yogurt can be influenced by the selection of a synergistic prebiotic source with a combination of bacteria in yogurt. The conclusion was obtained by the addition of *Bifidobacterium ssp* and *L. rhamnosus* Probiotics in synbiotics yogurt of yellow yam tuber is influence in maximizes the ability to inhibit pathogenic bacteria.

Keywords: simbiotic yogurt, probiotic, patoghenic bacteria

INTRODUCTION

Microbial cultures in food have 2 main roles namely "*food processing*" and "*product development*". "*Food processing*" is the role of microbes in food fermentation processes, fermentation control process is also no health benefits that can be generated whereas in "*product development*" refers to the functional role of microbes in foods that provide health benefits when consumed (Mani-Lopez *et al*, 2014). Yogurt is a product of fermented milk that uses lactic acid bacteria as its starter (Yelnetty and Tamasoleng, 2019). Yogurt products can contain two types of culture that are generally *S. Thermophilus*, *L. Bulgaricus* and starter of probiotics so that different bacterial combinations can be used to provide health and therapeutic benefits.

The presence of probiotic bacteria in yogurt can maximize the health benefits that will be gained when consuming yogurt (Madhu et al, 2012; Mani-Lopez et al, 2014). The ability to inhibit pathogenic bacteria is one of the health benefits gained when yogurt is consumed. Some probiotics that have been researched have the ability to inhibit pathogenic bacteria is *L. rhamnosus* and *Bifidobacterium ssp*. Based on this it is done research on the influence of the addition of the probiotic *L. rhamnosus* and *Bifidobacterium ssp* in sinbiotics tuber uwi yellow yogurt to the inhibitory ability of bacterial pathogens.

RESEARCH METHODS

Tools and Materials

Culture of lactic acid bacteria *L. rhamnosus*, *Bifidobacterium ssp*, *L. Bulgaricus*, *Streptococcus thermophilus*, *escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* acquired in Microbiology Laboratory of Department of Animal Husbandry Sam Ratulangi University, skim milk, a yellow yam tuber from Sitaro Indonesia, MRS Agar (De Man Rogosa and Sharpe), MRS Broth, NA (natrium Agar) and NB (natrium Broth).

Equipment used include thermometers, measuring pipettes, drip pipettes, analytical scales, measuring cups, laminar flow (LAF), incubators, petri cups, reaction tubes, 250 ml erlenmeyer, centrifugation, stirrer, Autoclaf, pH meters, and grinders.

Preparation of Yellow Yam Tuber Flour

Yellow Yam Tuber is peeled and cut thin. Soak in aquades for 3 hours before blanching with steam for 30 minutes. Dry at 56°C for 48 hours uses the oven. The slices of tuber be mashed use a grinder and sifted using the sieve size of 80 mesh (Yelnetty and Tamasoleng, 2019).

Preparation of Starter Culture

A total of 8% skimmed milk is pasteurized at 80°C for 15 minutes. Inoculate the bacteria that be used (*L. rhamnosus*, *Bifidobacterium ssp*, *L. Bulgaricus* and *S. Thermophilus*) each into 8% skim milk. Incubation at a temperature of 36°C for 18 hours (Yelnetty dan Tamasoleng, 2019).

Preparation of Symbiotic Yogurt

A total of 250 ml milk is added by 8% skimmed milk, 1% of tuber flour and 6% sugar and homogenized. Pasteurization of milk at a temperature of 80°C for 15 minutes and let in the container until the temperature of milk reaches 40°C. Inoculation starter yogurt in milk with a ratio of ST: LB: Probiotics (1:1:1) and incubation at 43°C for 6 hours. Yogurt is stored until the analysis is done (Yelnetty and Tamasoleng, 2019).

Total of Lactic Acid Bacteria (LAB) and pH

A total 1 ml of yogurt sample was put into a test tube containing a physiological solution to obtain a dilution of 10^{-1} . A total yogurt sample 1 ml is inserted from the first test tube into the second tube and a 10^{-2} dilution is obtained, continued until the specified dilution. The last dilution is entered into MRS Agar in petri dishes and homogenization. Let the MRS Agar stand until it is solid, then incubate at 36°C for 48 hours. The total bacteria that grows is calculated on MRS media according to the formula :

$$\text{Number of colonies per ml} = \Sigma \text{koloni} \times \frac{1}{\text{dilution factor}}$$

Analysis Inhibitory of Pathogenic Bacteria

A total 1 ml of pathogenic bacteria is taken and diluted to 10^{-7} in Natrium Agar. The 10^{-7} dilution results are divided into petri dishes as treated and let stand until the media becomes solid. A total of 3 wells were made on NA according to each treatment and 0.1 ml was added to the well according to each treatment. Incubation at 36°C for 24 hours. The diameter of the clear zone is calculated using the calipers in each well.

Data Design and Analysis

The research was designed uses Complete Random Design with 3 types of treatments, including 1 control and 3 replications. Data is analyzed with Anova test and continued Tukey test using Minitab 17 program. The treatment in the study as follows:

- R0: *S. Thermophilus*, *L. Bulgaricus*, yam tuber flour.
 R1: *S. Thermophilus*, *L. Bulgaricus*, *L. rhamnosus*, yam tuber flour.
 R2: *S. Thermophilus*, *L. Bulgaricus*, *Bifidobacterium ssp*, yam tuber flour.

RESULTS AND DISCUSSION

a. Total Lactic Acid Bacteria and pH of Yogurt

The results showed that the total lactic acid bacteria from yogurt increased after adding *L. rhamnosus* and *Bifidobacterium ssp* (Table 1).

Table 1. Total Lactic Acid Bacteria and Yogurt pH Value with Addition of Probiotics

Treatment	Total BAL (log cfu / ml)	pH
<i>S. thermophilus</i> and <i>L. bulgaricus</i>	9.50 ± 0.46 ^b	4.9 ± 0.05
<i>S. thermophilus</i> , <i>L. bulgaricus</i> and <i>L. rhamnosus</i>	9.60 ± 0.47 ^b	4.8 ± 0.05
<i>S. thermophilus</i> , <i>L. bulgaricus</i> and <i>Bifidobacterium ssp</i>	11.04 ± 0.14 ^a	4.8 ± 0.05

Note. Different letters show significant differences (P < 0.05)

These results indicate that the addition of probiotics affects the total lactic acid bacteria from yogurt. Based on the Tukey test it was found that the addition of probiotics *Bifidobacterium ssp* was significantly different (P < 0.05) with yogurt control and yogurt *L. rhamnosus*. The difference value in each yogurt because each bacterium has a different metabolic activity that produces different specific organic acids and have an effect on the interaction between one bacterium and another. That suggested yogurt with the addition of *L. rhamnosus* has a low total LAB because of the growth of *L. bulgaricus* and *L. rhamnosus* be hampered by *S. thermophilus* whose acidity level increases faster when fermented with other probiotic cultures (Rutella *et al*, 2016). It is different with added yogurt *Bifidobacterium ssp* which when cultured together with *S. thermophilus* and *L. bulgaricus* will increase the total amount of BAL yogurt in which amount *L. bulgaricus* more than *Bifidobacterium ssp* and *S. thermophilus* (Akalin *et al*,

2004). The bacterial interaction causes there are bacteria that are dominant in culture and bacteria that are not dominant in culture during the growth and development phase of bacteria. Based on the results of the study, symbiotic yogurt of yellow uwi tuber which was added by probiotics of *Bifidobacterium ssp* and *L. rhamnosus* in accordance with yogurt quality requirements where the minimum total number of bacteria was 6 - 8 log cfu / ml (FAO / WHO 2002).

b. Power of inhibiting pathogenic bacteria

In general, probiotic bacteria are known to produce organic acid compounds such as antibacterial and lactic acid (Sreekumar and Hosono, 2000). The ability of sinbiotic yogurt products to inhibit pathogenic bacteria is influenced by probiotic activity of bacteria in yogurt. This can be seen in the results of the study where after added probiotics there was an increase in the value of inhibition of pathogenic bacteria compared to continuous treatment (Figure 1).

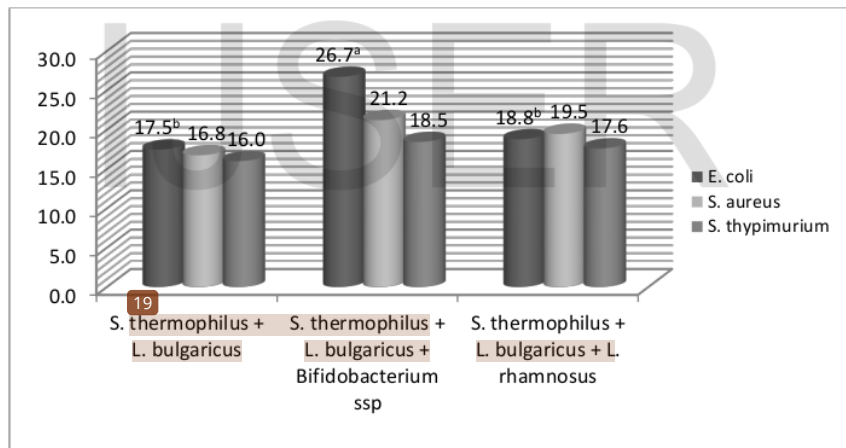


Figure 24 The Power of Inhibiting Sinbiotic Yogurt in Pathogenic Bacteria. Different letters show significant differences (P < 0.05).

The highest inhibition of *Escherichia coli* is found in yogurt with the addition of *Bifidobacterium ssp* as much as 26.7 mm. Tukey test result showed that the addition of *Bifidobacterium ssp* in simbiotic yogurt was significantly different (P < 0.05) with yogurt added *L. rhamnosus* and control yogurt. That suggested the mechanism of inhibition of *Escherichia coli* due to increased acid,

acetic acid and acid bacteria laktat so the environment becomes acidic, and causing *Escherichia coli* will not be able to survive in these environmental conditions. The growth of *Bifidobacterium ssp* will be superior (Ibrahim and Bezkorovainy, 1993). The inhibitory mechanism occurs because there are protonation (not dissociated) ions from acetic acid and lactic acid that penetrate the cell plasma membrane into the core part of the cell resulting in the dissociation of acetic acid and lactic acid to form protons which will inhibit the work of cellular pathogenic bacteria (Sharma *et al*, 2017) .

The highest inhibition of *Staphylococcus aureus* found in yogurt with the addition of *Bifidobacterium ssp* amounted to 21.2 mm and highs against *Salmonella typhimurium* are also on the inhibition of *Bifidobacterium ssp* amounted to 17.6 mm. The previous study also stated that probiotics *Bifidobacterium ssp* has inhibitory activity against *Staphylococcus aureus* (Zarate and Nader-Macias, 2006; Zarate and Nader-Macias , 2007) and *Salmonella typhimurium* (Vazquez-Gutierrez *et al* , 2016). This occurs because of differences in the level of adhesion in bacterial epithelial cells (Lazarenko *et al* , 2012). Antimicrobial compound produced by *Bifidobacterium ssp* will inhibit the ability of pathogens to secrete intracellular compounds and reducing the level of adhesion of pathogenic bacteria to the intestinal epithelial cells (Botes *et al*, 2008; Xu *et al*, 2009).

Yogurt added by *L. rhamnosus* also showed inhibition of pathogenic bacteria but not as much as the inhibitory value of *Bifidobacterium ssp*. Previous research states that the use of *L. rhamnosus* does not show the inhibiting effect of pathogenic bacteria (Calderon *et al*, 2007; Sharma *et al*, 2017). Other studies stated that *L. rhamnosus* has a value of inhibition <11 mm against *Staphylococcus aureus* bacteria (Sharma *et al*, 2017).

The results showed that the inhibition of pathogenic bacteria by symbiotic yogurt yellow yam tuber was caused by metabolic activity of probiotics which influenced the growth of pathogenic bacteria and the presence of antibacterial compounds produced by probiotics used in starter culture combinations. Previous research stated that the inhibition of pathogenic bacteria is influenced by the

metabolism of probiotics in producing organic acids, as like bacteriocin, active protein under acidic conditions (Millette *et al* , 2007). The difference in the inhibitory value of sinbiotic yogurt is thought to be caused due to differences in the secretion of antimicrobial or metabolite substances such as organic acids (lactic acid and acetic acid), hydrogen peroxide, ethanol, diacetyl, bacteriocin and peptides in each probiotic (Kumar and Kumar, 2015), especially in every combination of bacteria in yogurt. The presence of bacterial interactions can affect the ability of the yogurt to provide health effects when consumed.

In addition, symbiotic yogurt inhibition of pathogenic bacteria is also caused by the availability of specific substrates for fermentation by probiotics. The metabolic activity of probiotics is influenced by the presence of prebiotic sources. Previous research stated that *L. rhamnosus* only had inhibitory activity amount <11 mm (categorized as no inhibitory activity) (Sharma et al, 2017), presumably because there was a synergism between probiotic bacteria and prebiotics, the inhibitory value increased with increasing metabolic BAL compounds. This is evidenced by the increasing value of inhibition of pathogenic bacteria by symbiotic yogurt *L. rhamnosus* which shows better activity amount > 11 mm.

Overall symbiotic yogurt added by probiotics *L. rhamnosus* and *Bifidobacterium ssp* gave a synergistic effect with 1% yellow yam tuber flour. This is shown in the results of the study where the total value of LAB and the inhibitory value of pathogenic bacteria increased. Previous research on the concentration of uwi tuber flour in probiotic drinks also stated that 1% yam tuber flour can increase and maximize the activity of probiotic bacteria (Yelnetty and Tamasoleng, 2019). Yellow yam tuber flour have a lot of inulin compound which can stimulate growth, all the products of the main BAL metabolites (Oliveira *et al*, 2012) especially organic acids and bacteriocins which are responsible for the inhibitory ability of pathogenic bacteria. The Yellow yam tuber flour has inulin compound amounting to 1.52 mg / g (Winarti and Saputro, 2013).

CONCLUSION

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The total of Lactic Acid Bacteria (BAL) and value inhibition of pathogenic bacteria by yogurt symbiotic is influenced by the combination of bacteria used. The synergistic relationship 25 between the combination of lactic acid bacteria, additional probiotics and the prebiotic source used also has an effect on maximizing the desired therapeutic effect. Addition of probiotics *L. rhamnosus* and *Bifidobacterium ssp* in symbiotic yogurt that uses 1% yellow yam tuber flour as a prebiotic source can provide good health benefits, especially in inhibiting the growth of pathogenic bacteria.

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