Enhancing Anti-pathogenic Bacteria Activity of *Lactobacillus Plantarum* AKK-30 Cultured on the Medium Containing Fructose-Oligosaccharides

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Abstract: The purpose of this study was to evaluate the concentration of Fructose-Oligosaccharides (FOS) in correlation with incubation time for growing - *Lactobacillus plantarum* AKK-30, and to assess metabolites of *L. plantarum* AKK-30 as antimicrobial substances against to pathogenic bacteria. *L. plantarum* AKK-30 was isolated from the small intestine of native chicken. *L. plantarum* AKK-30 was grown on MRSB medium containing FOS (0%, 0.5%, 1%, and 1.5%), and incubated at different times (6, 12, 18, and 24 hours) at 37°C. Antimicrobial activity of *L. plantarum* AKK-30 metabolites was tested on four species of pathogenic bacteria consisted of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella pullorum*. The results showed that the concentration of 1% FOS and 24-hours incubation were most effective in increasing *L. plantarum* AKK-30 growth (2.11 x 10^8 CFU/ml). Antimicrobial activity extract of *L. plantarum* AKK-30 metabolites was able to inhibit the growth of *E. coli, P. aeruginosa, S. aureus*, and *S. pullorum*. The highest inhibition of bacteria was observed on *S. aureus* which was 10.8 mm, followed by *E. coli* at 9.9 mm, *S. pullorum* at 9.083 mm, and *P. aeruginosa* 8.783 mm.

1 INTRODUCTION

Lactobacillus plantarum AKK-30 is a lactic acid bacteria (LAB) isolated from Indonesian native chicken (Damayanti *et al*., 2014) and has been identified microbiologically, biochemically, and molecularly (Istiqomah *et al*., 2017). This species reported that has an activity of enzyme cholesterol reductase (Julendra *et al*., 2017; Palaniyandi *et al*., 2019). *L. plantarum* AKK-30 has an inhibitory agent for pathogenic bacteria and produces antimicrobials (Julendra *et al*., 2018; Sophian *et al*., 2018) and could be used as probiotics for poultry (Wei *et al*., 2018).

Previous studies have reported that potency antibacterial activity of *L. plantarum* (Kabir *et al*., 2009), (Yang *et al*., 2017), (Lin and Tzu-M, 2017). The growth of probiotic bacteria can be increased by the addition of oligosaccharides in their medium (Pranckute *et al*., 2016). The use of inulin and mono-oligosaccharides as prebiotics has been investigated for increasing viability of *L. plantarum* AKK-30 (Julendra *et al*., 2018). However, Julendra *et al.* (2018) reported that a combination of *L. plantarum* AKK-30 and oligosaccharides were not significant influences of antibacterial activity. Addition of mannan oligosaccharides (MOS) at 0.5 - 2% could increase *L. plantarum* AKK-30 growth with 0.5% MOS. However, the possibility of improving *L. plantarum* growth by combining FOS has not been reported. FOS is an oligosaccharide composed of 2-10-unit fructose monomers with bonds -(2-1) glycoside and one glucose monomer with bonds -(2-1) glycoside at the ends (Yuliana *et al*., 2014). Addition of FOS in probiotics was for microbial nutrition (Setiarto *et al*., 2017), it could improve metabolism of probiotic bacteria and increase the number of bacterial cell biomass, bacteriocin increases (Ogunbanwo *et al*., 2003), inhibited the growth of pathogenic bacteria (Pranckute *et al*., 2016).

Addition of FOS prebiotic in media is expected to stimulate the growth of *L. plantarum* AKK-30 because a FOS was more soluble than inulin (about 80% in water at room temperature) (Gibson *et al*., 2017). Therefore, a current study was conducted to evaluate the effect of FOS addition in growth medium

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on enhancing the anti-pathogenic bacteria activity of *L. plantarum* AKK-30.

2 MATERIALS AND METHODS

2.1 Materials and Research Design

The research was conducted at the Research Division of Natural Product Technology (BPTBA)-Indonesian Institute of Sciences (LIPI) at the Microbiology Laboratory, from November to December 2018 using *L. plantarum* AKK-30 isolates belonging to the BPTBA-LIPI Microbiology Laboratory and the commercially obtained of Fructo-oligosaccharides (FOS). FOS was dissolved in distilled water until homogeneous and then sterilized using 0.22 µm Millipore then implanted in MRSA. 2 mL of MRSB and 1% of *L. plantarum* AKK-30 were added into microtube and then were vortexed and incubated 24 hours at 37ºC. A series of falcon tubes filled with 10 mL of sterile MRSB were added with FOS (0%, 0.5%, 1%, 1.5%) and 1% of *L. plantarum* AKK-30 culture (Setiarto *et al*., 2017). Pathogenic bacteria used were *Escherichia coli* FNCC 0194, *Staphylococcus aureus* FNCC 6049, *Pseudomonas aeruginosa* FNCC 0063, and *Salmonella pullorum* ATCC 13036, the bacteria were grown in nutrient agar (NA) [Merck].

The study used an experimental method with two stages using a Factorial-Completely Randomized Design (FCRD). The experiment was arranged using two stages; the first stage was optimization of *L. plantarum* AKK-30 growth added with FOS 0%, 0.5%, 1%, and 1.5% and the second stage using a Completely Randomized Design (CRD) tested the antimicrobial activity of metabolites from the best growth results of *L. plantarum* AKK-30 in the first stage of which each treatment consisting of 4 replications. The parameters measured were bacterial growth and antibacterial activity.

2.1.1 Total Plate Count (TPC)

Colonies of *L. plantarum* AKK-30 were enumerated by the TPC method as previously reported by Setiarto *et al*. (2017). Briefly, 1 ml of *L. plantarum* AKK-30 culture (6, 12, 18 and 24 hours) was diluted with 9 ml of sterile distilled water until 10^{-7}. 1 ml of culture was inoculated on MRSA by pour plate method, then incubated at 37°C for 48-hours.

2.1.2 Antimicrobial Activity

Antimicrobial activity was assessed according to Damayanti *et al.* (2014). Briefly, *L. plantarum* AKK-30 culture was centrifuged at 12.500 rpm and 4°C for 15 minutes. The supernatant was neutralized using 0.5N NaOH and sterilized using 0.22 µL millipore. Agar diffusion method (Bonev *et al*., 2008) was used to test the inhibitory activity of pathogenic bacteria by inoculating pathogenic bacteria on NA media (Merck) and 50 µL supernatant dripped on sterile disk paper. Then incubated for 24-hours at 37°C. Positive results of antimicrobial activity were revealed by the formation of clear zones around the disk paper.

2.2 Data Analysis

Quantitative data from bacterial growth and antibacterial activity were analyzed by using analysis of variance (ANOVA) and followed by Duncan's multiple range test to distinguish the effect of different treatment mean using CoSTAT statistical software (Cohort, 2008).

3 RESULT AND DISCUSSION

3.1 The Growth of *Lactobacillus plantarum* AKK-30

The results of the growth *L. plantarum* AKK-30 with the addition of different FOS in MRSA were presented in Figure 1.

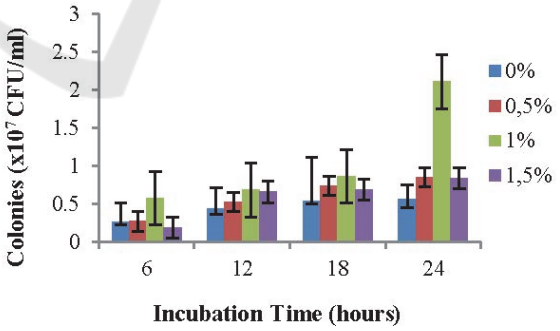

Figure 1: Growth of *Lactobacillus plantarum* AKK-30 at different FOS and incubation times.

The results showed that the growth of *L. plantarum* AKK-30 with FOS 1% better than other treatments, it was seen starting from 6-hours of incubation and the highest number of bacterial colonies of *L. plantarum* AKK-30 (2.11 x 10^8 CFU/ml) occurred when 24-hours incubation. It was

explained that the growth of *L. plantarum* AKK-30 was influenced by the addition of 1% FOS and significantly different (P <0.05) with no addition of FOS. The interaction between FOS and incubation time effect on *L. plantarum* AKK-30 growth was explained in Table 1.

Table 1: Interactions Between FOS Concentration and Incubation Time (Log CFU/mL).

FOS (%)	Time of Incubation (hours)			
	6	12	18	24
0	7.411 ± 0.052 [a]	7.639 ± 0.059 [b]	7.722 ± 0.129 [bc]	7.738 ± 0.117 [bc]
0.5	7.417 ± 0.140 [a]	7.718 ± 0.03 [bc]	7.886 ± 0.129 [bc]	7.929 ± 0.040 [c]
1.0	7.758 ± 0.03 [bc]	7.834 ± 0.009 [bc]	7.926 ± 0.129 [c]	8.261 ± 0.274 [e]
1.5	7.225 ± 0.249 [a]	7.815 ± 0.068 [bc]	8.039 ± 0.129 [d]	7.736 ± 0.223 [bc]

[a,b,c,d]: Means in the same column and row differ significantly (P<0.05).

The results showed that at the 24-hour incubation of *L. plantarum* AKK-30 with the addition of FOS were as follows FOS 0% (7.738 ± 0.117), FOS 0.5% (7.929 ± 0.040), FOS 1% (8.226 ± 0.274) and FOS 1.5% (7.736 ± 0,223). At 24-hour incubation, growth of *L. plantarum* AKK-30 adding 1% FOS was significantly higher (P<0.05) (8.226 ± 0.274) than other treatments.

3.2 Antibacterial Activity

The antibacterial in *Lactobacillus* is obtained from its metabolite compounds (Setiarto *et al*., 2017) called bacteriocin (Rawal *et al*., 2013).

Table 2: The Diameter of Inhibition Zones of *L. plantarum* AKK-30 with FOS 1%.

Metabolic Extract	The Diameter of Inhibition (mm)			
	S. aureus	*P. aeruginosa*	*S. pullorum*	*E. coli*
L. plantarum AKK-30	10.8 [b]	8.783 [a]	9.083 [ab]	9.9 [ab]

[a,b]; Means in the same column differ significantly (P<0.05).

In Table 2, *L. plantarum* AKK-30 with FOS 1% demonstrated antibacterial ability as evidenced by the inhibition zone in the growth of pathogenic bacteria, *Staphylococcus aureus* FNCC 6049, *Pseudomonas aeruginosa* FNCC 0063, *Salmonella pullorum* ATCC 13036 and *Escherichia coli* FNCC 0194. The antibacterial activity of *L. plantarum* AKK-30 against *Staphylococcus aureus* was significantly higher (P<0.05) of 10.8 ± 3.59 compared to other pathogenic bacteria. In Table 2, it can be said that the bacteriocin in *L. plantarum* AKK-30 has inhibited Gram-positive or Gram-negative bacteria. Bacteriocin has a broad spectrum and could inhibit the growth of pathogenic bacteria (Sifour *et al*., 2012; Arief *et al*., 2013; Khikmah, 2015; Sulistiani, 2017).

The bacteriocin was an extracellular protein that has antimicrobial activity (Sari et al., 2018). Mechanism of inhibition of microbial growth by bacteriocin is the cell wall damage and have causing lysis (Pranckute *et al*., 2016), the cell's metabolic system was disrupted by inhibiting the activity of intracellular enzymes (Pelczar and Chan, 1998) and disruption of cytoplasmic membrane permeability (Hasan and Wikandari, 2018).

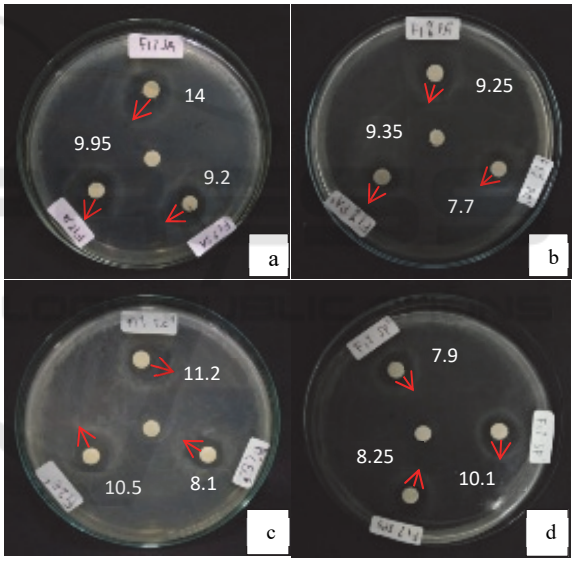

Figure 2: Inhibitory Activity Metabolic Extracts of *L. plantarum* AKK-30 (mm) (a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa* (c) *Escherichia coli* and (d) *Salmonella pullorum*.

In Figure 2. it was shown that all pathogenic bacterial growth was inhibited by *L. plantarum* AKK-30 metabolite, the widest inhibitory zone was *Staphylococcus aureus* and the lowest was *Pseudomonas aeruginosa*. Inhibition zones were influenced by bacteriocin concentrations (Julendra *et al*., 2018), bacteriocin activity (Forte *et al*., 2016), types of lactic acid bacteria (Kasi *et al*., 2017) and different bacterial lipid layers (Jawetz *et al*., 2005), (Radji, 2011). The active substance in bacteriocin from *L. plantarum* was plantaricin (Gonzalez *et al*.,

1996), antibacterial (Lim *et al*., 2007) that could lysis cell membranes of pathogenic bacteria (Lu *et al*., 2017).

The difference in width of the inhibition zone can be caused by the bacterial lipid layer (Radji, 2011). Gram-negative bacteria have thin peptidoglycan but there are three polymers outside of peptidoglycan namely lipoprotein, outer membrane, and lipopolysaccharide. Permeable outer membranes are resistant to low molecular weight substances and hydrophilic solutes but are relatively quickly penetrated by high molecular weight substances such as bacteriocin (Jawetz *et al*., 2005). The mechanism of bacteriocin is to damage the cell wall causing lysis (Hasan and Wikandari, 2018), and inhibit cell wall growth, change the permeability of cytoplasmic membranes, denaturation of cell proteins, and damage the metabolic system (Pelczar and Chan, 1998).

The action of plantaricin, in inhibition of *Staphylococcus aureus* is by blocking the permeability of the cytoplasmic membrane and inducing the release of Adenosine Triphosphate (ATP), chloroplast factor (CF), and glutamate (Gonzalez *et al*., 1996). Plantaricin can disrupt cell membranes of Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* (Lu *et al*., 2017), and causes the release of intracellular components of enzymes and ions (Lim and Im., 2007).

4 CONCLUSIONS

The highest total plate count of *L. plantarum* AKK-30 was found at the medium containing 1% FOS with 24-hour incubation. The highest inhibition of *L. plantarum* AKK-30 was observed against *Staphylococcus aureus* (10.8 mm), followed by *Escherichia coli* (9.9 mm), *Salmonella pullorum* (9.083 mm), and *Pseudomonas aeruginosa* (8.783 mm).

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