THE ABILITY OF *LACTOBACILLUS PLANTARUM* BSL IN REDUCING THE TISSUE DAMAGE OF LIVER AND SPLEEN IN RATS INFECTED BY *LISTERIA MONOCYTOGENES* ATCC 7644

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Abstrak

Lactobacillus plantarum BSL merupakan hasil isolasi dari asinan kubis. Tujuan dari penelitian ini adalah untuk mengevaluasi kemampuan *L. plantarum* BSL dalam mereduksi kerusakan jaringan hati dan limpa pada tikus yang diinfeksi oleh *Listeria monocytogenes* ATCC 7644. Kelompok perlakuan tikus menerima 0,5 kultur suspensi (10^9 CFU/mL) *L. plantarum* BSL dan kelompok kontrol menerima 0,5 mL NaCl 0,85% setiap hari selama sembilan hari percobaan. Kedua kelompok tikus diinfeksi pada hari ke-3 dengan 0,5 mL kultur suspensi (10^9CFU/mL) *L. monocytogenes*. Pada hari ke-2 (sebelum diinfeksi), ke-5, ke-7, dan ke-9 (setelah diinfeksi), tikus dibedah. Selanjutnya, hati dan limpa tikus diuji secara histologi. Hasil penelitian menunjukkan bahwa pemberian *L. plantarum* BSL dapat mereduksi kerusakan hati dan limpa tikus selama percobaan.

Kata kunci: *Lactobacillus plantarum* BSL; *Listeria monocytogenes*; Probiotik

Abstract

Lactobacillus plantarum BSL, previously isolated from Indonesian sauerkraut. In this study, we investigated the ability of L. plantarum BSL in reducing the tissue damage of liver and spleen in rats infected by Listeria monocytogenes ATCC 7644. Treatment group of rats received 0.5 mL culture suspension (10^9 CFU/mL) of L. plantarum BSL and control group received 0.5 mL of 0.85% w/v NaCl daily for nine days of experiment. Both groups were infected at 3rd day with 0.5 mL of suspension of L. monocytogenes (10^9CFU/mL). At the 2nd (before infection), 5th, 7th, and 9th day (after infection), the rats were sacrificed and then, liver and spleen were assessed for histopathological. Our study revealed that the administration of L. plantarum BSL could be able to reduce the liver and spleen damage of the experimental rats.

Keywords: *Lactobacillus plantarum BSL; Listeria monocytogenes; Probiotic*

1. INTRODUCTION

Listeria monocytogenes is a foodborne pathogen which may lead to serious clinical diseases with a mortality rate of 20% - 30% (Lee *et al*. 2014; Jordan & McAuliffe 2018). Listeriosis is a series of diseases caused by the bacteria *L. monocytogenes*. There are two main types of listeriosis: a non-invasive form and an invasive form. Non invasive listeriosis (febrile listerias gastroenteritis) is a mild form of the disease affecting mainly otherwise healthy people. Symptoms include diarrhea, fever, headache and myalgia. Invasive listeriosis is a more severe form of the disease and affects certain high risk groups of the population. These include pregnant women, patients undergoing treatment for cancer, AIDS and organ transplants, elderly people and infants (WHO 2018; Tebib *et al*. 2018). Cases of listeriosis result from consumption of contaminated food, particularly ready-to-eat foods, such as dairy products, smoked fish, and seafood (Allen *et al*. 2016).

Probiotics are live microorganisms which when administered in adequate amounts confer

a health benefit to the host (FAO/WHO, 2002a).The positive effects of probiotics are modulation of immune function, protection from diabetes mellitus, prevention of asthma, protection of barrier function in intestinal epithelial cells, anti-allergy and also as ananti-inflammatory (Gobbetti and Minervini 2014; Yu *et al*. 2017).

Lactobacillus plantarum BSL isolated from sauerkraut has resistance to 5% bile salt and pH 2.5. *L. plantarum* BSL also has antagonistic properties against pathogen such as *B. cereus*, *S. aureus*, and *E. coli*. Moreover, *L. plantarum* BSL were able to reduce total cholesterol of rat blood serum, enhance lactobacilli growth, and suppress coliform and staphylococci in rat faeces (Kusumawati *et al*. 2003; Kusumawati *et al.* 2008). In addition, Meiyasa *et al*. (2017) reported that administration of *L. plantarum* BSL significantly increased the population of LAB and reduced the number of *L. monocytogenes* compared to control group either in the faeces, caecum, or caecum content, and it effectively reduced the liver and spleen tissues damage in rats. Numerous studies have reported that probiotics have a beneficial effect on the host. However, the role of probiotics in reducing tissue damage of liver and spleenin rats infected by *L. monocytogenes* little has been reported.

Based on this, that study aims to evaluate the ability of *L. plantarum* BSL in reducing tissue damage of liver and spleen in rats infected by *L. monocytogenes*.

2. MATERIAL AND METHODS

2.1 Rats

Sprague-Dawley rats (*Rattus norvegicus*) were purchased from Faculty of Animal Science, IPB University. A total of 60 rats (40days old) were randomly allocated and housed in cages (one rat per cage). The rats were housed under standard conditions in the barrier unit: light/dark schedule was constant at 12/12 h and humidity at 50– 60% (de Waard*et al*. 2002). All experiments were approved by Animal Ethical Committee of Bogor Agricultural University.

2.2 Diets

Diet of 20 g/rat/day of feed was formulated according to AOAC (2005) consisted of casein, corn oil, mineral mix, vitamin mix, carboxymethyl cellulose (CMC), water, and cornstarch.

2.3 Bacterial Culture

L. plantarum BSL was obtained from Laboratory of Food Microbiology, Department of Food Science and Technology, IPB University. *L. plantarum* BSL was grown in MRS broth (Oxoid CM359) at 37 °C for 24 h, followed by pour plating in MRS agar (Oxoid CM361) incubated at 37 °C for 24-48 h for counting the bacterial cells. *L. monocytogenes* strain ATCC 7644 was cultured BHI broth (Oxoid CM1136) incubated at 37 °C for 24 h, and then counted in Listeria Selective Agar Base (Oxoid CM856), after incubation at 37 °C for 24-48 h (Meiyasa *et al*. 2018).

To prepare cell suspension, a 24 h culture of *L. plantarum* BSL in MRSB was centrifuged (2000 g, 10 min, 4 °C) and the cell pellet was resuspended in 10 mL volume of 0.85% NaCl. The number of cells of *L. plantarum* BSL in suspension was enumerated in MRS agar. Similar method was applied for *L. monocytogenes* ATCC 7644, only the media was used BHI broth (Meiyasa *et al*. 2018).

2.4 In *vivo* experiment of antilisterial activity (de Waard *et al*. 2002)

Rats having initial weight of 100-140 g were acclimatized for 1 week and received a standard diet. In anti-listerial experiment, a total of 48 rats were divided into 2 groups (24 rats for treatment group and 24 rats control group).

L. plantarum BSL and *L. monocytogenes* ATCC 7644 were grown in MRS broth and BHI broth respectively for 24 h at 37 °C. This culture were centrifuged (2000 g, 10 min. 4 °C) and resuspended in 0.85% NaCl in order to obtain cell number up to 10^9 CFU/mL. This suspension was then administered to rats by the oral route.

The treatment group was administered with*L. plantarum* BSL culture (10^9 CFU/mL), while the control group was administered with 0.85% NaCl (0.5 mL/rat/day) for 9 days (day-0 – day-9, except at day-3). Rats were then infected by 0.5 mL of *L. monocytogenes* ATCC 7644

The rats were sacrificed by cervical dislocation in 2^{nd}day (before infected by *L. monocytogenes*), 5^{th} day, 7^{th} day, and 9^{th} day (after infected by *L. monocytogenes*) to obtain for histopathology analysis, rat spleen and liver

were stained using Hematoxylin-Eosin (HE) and observed the cell changes under light microscope. Data were evaluated using the descriptive analysis.

3. RESULTS AND DISCUSSION

Histological analysis on liver such asperivascular macrophages (PVM), fatty degeneration (FD), sinusoidal dilatation (SD), and central venous of vibrosis (CVV). Based on the research results show that descriptively administration of *L. plantarum* BSL could reduce tissue damage of liver compared with the control group (Table 1). It is seen that before rats infected by *L. monocytogenes* (in day-2) there was no change in the PVM and FD. However, there has been a change on the SD and CVV in rats liver either in control or treatment group.

Administration of *L. plantarum* BSL for nine days after rats infected by *L. monocytogenes* showed that could be able to reduce the level of damage in rats liver tissue, especially in the CVV. On the other hand, tissue damage of rats liver in the control group were still observed with the level of minor (+) and moderate (++) damage approximately 67% of the rat population (4/6). In addition, administration of *L. plantarum* BSL could be able to reduce damage of rats liver such as, PVM and FD approximately 33% of the rat population (2/6) and SD of about 17% of the rat population (1/6) with extent of minor damage (+). In contrast, control group at ninth day were still observed and even more severe as PVM and FD with damage level minor (+), moderate (++) and heavy (+++) approximately 67% of the rat population (4/6). Furthermore, SD with the extent of damage minor (+) and moderate (++) approximately 50% of the rat population (3/6).

Spleen damage in rat was observed including lymphoid glands (LG), increased macrophage cells (IMC), secondary lymphoid cells (SLC) and increased giant cells (IGC) (Table 2). Damage of rat s pleen also observed as lymphoid glands, increased macrophage cells, secondary lymphoid cells and increased giant cells were detected at two days, before infected with *L. monocytogenes* in both the control and the experiment group. Administration of *L. plantarum* BSL for nine days could be able to reduce spleen tissue damage in rats compared with the control group. For nine days of treatment with *L. plantarum* BSL could be able to reduce tissue damage in rats spleen. However, tissue damage in rats spleen were still observed at nine days.

In rats, administered with *L. plantarum* BSL, five rats (5/6) appear normal again either in the LG, SLC, IMC or IGC with one of six rats suffered minor damage (+). In contrast to the treatment group, in the control group most of the rats were damaged. Seen in the LG and SLC with extent of the damage minor (+) and moderate (++) approximately 67% of the rat population (4/6). Moreover, IMC and IGC also increased by about 50% of the rat population (3/6). Based on this, it can be concluded that the administration of *L. plantarum* BSL for nine days was able to reduce tissue damage of ratsliver and spleen compared with the control group.

In agremment with a previous study reported by Bambirra *et al*. (2007), that treatment with *Lactobacillus sakei* 2a could be able to reduce damage lesions and also destruction of the mucosa with infiltrate of inflammatory cells, predominantly macrophages and neutrophil in rats infected by *L. monocytogenes* Scott A. Furthermore, dos Santos *et al*. (2011) reported that treatment with *L. delbrueckii* UFV-H2b20 significantly reduced the damage of liver such as necrosis and degenerative changes of hepatocytes lower than the control group in rats infected by *L. monocytogenes* 10403S. Other than that, *L. plantarum* B7 was able to reduce liver translocation and ameliorates histopathological lesions in rats infected by *S. thphimurium* (Tsai *et al*. 2005), and then Acurcio *et al*. (2017) also reported that administration of *L. plantarum* B7 significantly extent of ileal villi heights and number of hepatic inflammatory foci significantly lower than the control group. In addition, Meiyasa (2017) reported that treatment with *L. plantarum* BSL during nine-days could be able to reduce damage of liver and spleen in rats such as edema, necrosis, cell infiltration of lymphoid, proliferation of bile tract, vacuolization of hepatocytes cell for liver and depletion of lymphoid cells, extension of pulp area, and cell proliferation for spleen.

Administration of *L. plantarum* BSL can reduce tissue damage of liver and spleen in rats possibly through mechanisms such as the production of antagonistic compounds and

inhibitory substances, protective effects against pathogens (Acurcio *et al*. 2017; Servin 2004; Prieto *et al*. 2015 competition for nutrients or adhesion sites and/or immunomodulation (Bambirra *et al*. 2007; Gibson *et al*. 2005), cytokine production (Castanheira *et al*. 2007), and reduces pro-inflammatory interleukin secretion (Puertollano *et al*. 2008).

4. CONCLUSION

In summary, our findings demonstrate that *L. plantarum* BSL is able to protect the rats that associated with the ability in reducing the tissue damage of liver and spleen in rats which infected by *L. monocytogenes*.

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Table 1. Reduction of liver damage during nine-day administration of *L. plantarum* BSL.

Group	Number of rats	Days															
		2				5				7				9			
		Liver															
		PVM	FD	SD	CVV	PVM	FD	SD	CVV	PVM	FD	SD	CVV	PVM	FD	SD	CVV
Control	1	-	-	-	-	+	++	-	+	-	-	+	+	-	-	-	+
	2	-	-	-	-	-	+	-	+	+	++	++	-	++	+++	++	-
	3	-	-	-	-	+++	-	-	-	++	++	+	++	+++	++	++	++
	4	-	-	-	-	+++	++	++	-	+	+	-	-	+	-	-	+
	5	-	-	-	+	+	-	+	++	+++	+	+	+	+	++	+	-
	6	-	+	-	+++	+	+++	++	++	+	+++	-	+	-	++	-	+
Treatment	1	-	-	-	-	-	-	++	+	++	-	+	-	-	-	-	-
	2	-	-	-	-	+++	+++	+	-	++	++	-	-	-	-	-	-
	3	-	+	-	-	++	-	-	+	-	++	-	+	+	-	-	-
	4	-	++	-	-	+++	++	+	++	+	+	-	-	+	+	-	-
	5	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
	6	-	-	-	-	+	-	+	+	++	-	-	-	-	+	+	-

Note: Damage level of liver tissue: no (-); minor (+); moderate (++); heavy (+++)

Table 2. Reduction of spleen damage during nine-day administration of *L. plantarum* BSL

Group	Number of rats	Days															
		2				5				7				9			
		Spleen															
		LG	IMC	SLC	IGC	LG	IMC	SLC	IGC	LG	IMC	SLC	IGC	LG	IMC	SLC	IGC
Control	1	-	-	-	-	++	++	-	-	-	+	+	++	+	++	-	-
	2	-	-	-	-	-	-	-	-	++	++	++	+++	+	-	++	++
	3	+	-	-	-	+	++	-	+	++	++	+	-	-	++	+	+
	4	-	-	-	-	-	-	++	+++	-	-	-	-	++	-	+	-
	5	-	-	-	-	++	+	+	+	+	+++	+	+	+	-	++	++
	6	-	+	-	-	-	-	++	+	-	-	-	+	-	++	-	-
Treatment	1	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-
	2	-	-	-	-	-	+	-	++	+	-	-	-	-	-	-	-
	3	-	+	-	-	+	-	++	-	-	+	+	+	-	-	-	-
	4	-	-	-	-	-	++	++	++	-	-	+	-	-	-	-	-
	5	-	-	-	-	-	-	+++	-	-	+	++	+	-	-	-	+
	6	-	++	-	-	++	+	-	+	-	-	-	+	+	+	-	-

Note: Damage level of spleen tissue: no (-); minor (+); moderate (++); heavy (+++)