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Effectivity of *Lactobacillus plantarum* BSL against *Listeria monocytogenes* in rats

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ABSTRACT

Aims: To evaluate the effectivity of *Lactobacillus plantarum* BSL isolated from Indonesian sauerkraut against *Listeria monocytogenes* ATCC 7644 through *in vitro* and *in vivo* assay.
Methodology and results: *In vitro* examination for antimicrobial activity against *L. monocytogenes* ATCC 7644 was performed using seven isolates of lactic acid bacteria (LAB). *Lactobacillus plantarum* BSL demonstrated the highest activity against *L. monocytogenes* and studied further in Sprague-Dawley (SD) rats. 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 during nine days of treatment. Both groups were infected at 3rd day with 0.5 mL of suspension of *L. monocytogenes* (10^9 CFU/mL). At the 2nd (before infection), 5th, 7th, and 9th day (after infection), the rats were sacrificed and the faeces, caecum, and caecum content were examined for the population of LAB and *L. monocytogenes*. Administration of *L. plantarum* BSL significantly increased the population of LAB by 1.2–1.4 log unit, while the number of *L. monocytogenes* was reduced by 1.8–1.9 log unit compared to control group eithr in the faeces, caecum, or caecum content. Administration of *L. plantarum* BSL could be able to reduce the liver and spleen damage of the experimental rats, but did not show any changes in immunoglobulin A (IgA) response in comparison with control group.
Conclusion, significance and impact of study: *Lactobacillus plantarum* BSL was promising as probiotic candidate with health promotion to protect the gastrointestinal from infection by *L. monocytogenes* ATCC 7644.

Keywords: Lactic acid Bacteria*, L. plantarum BSL, L. monocytogenes,* probiotic, anti listerial

INTRODUCTION

Listeria monocytogenes is a foodborne pathogen, and its infections are primarily due to the ingestion of contaminated food-products (Lomonaco *et al*., 2009), which may lead to serious clinical diseases (Lee *et al*., 2014). *Lactobacillus monocytogenes* may cause gastroenteritis, neural infections, and spread through the blood stream, especially in immunocompromised patients and elderly individuals. Furthermore, gestating women were highly prone to *L. monocytogenes* infections, leading to premature birth, abortion, stillbirth, and serious health problems for newborn babies (CDC, 2011; Carpentier and Clerf, 2011). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2002a). As one of the beneficial effects, the presence of probiotics provides protection against gastrointestinal pathogens (Lourens-Hattingh and Viljoen, 2001). Several *in vitro* studies had reported that probiotics showed antagonistic activity against pathogens such as *L. monocytogenes* (Tulini *et al*., 2013; Huang *et al*., 2015; Leite *et al*., 2015).

In addition, several *in vivo* studies reported that probiotics from different species of *Lactobacillus* play an important role as antilisterial agent. *L. casei* significantly reduced the number of *L. monocytogenes* in the stomach, caecum, faeces, spleen and liver of the infected rats. It has also been reported that *L. casei* was able to increase cellular immunity in delayed-type hypersensitivity response (de Waard *et al*., 2002). Bambirra *et al*. (2007) further reported that *Lactobacillus sakei* 2a was able to survive in the mammal digestive tract and showed a protective effect against *L. monocytogenes*. *L. delbrueckii* UFV-H2b20 protected mice from death caused by *L. monocytogenes* infection and induced a faster clearance of bacteria in the liver, spleen and peritoneal cavity post-infection (dos Santos *et al*., 2011). This strain could produce high level of TNF-α in serum, peritoneal cavity and gut and increase the production of nitric oxide in serum and induced higher production of IL-10 in the

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mucosal immune system. High level of TNF-α and IL-10 will improve the immune system

Indigenous lactic acid bacteria (LAB) had been widely isolated from various Indonesian fermented foods among others were fermented vegetables, fruits, legumes, fish and fresh beef. One promising LAB strain (*L. plantarum* BSL), isolated from sauerkraut was considered as a good probiotic candidate due to its ability to survive the gastric environment (5% bile salt and pH 2.5) and showed significant health benefit. *L. plantarum* BSL demonstrated inhibitory activity against pathogens such as *B. cereus*, *S. aureus*, and *E. coli* (Kusumawati *et al*., 2003) and *in vivo* experiment revealed that the LAB was able to reduce total cholesterol of rat blood serum, enhance lactobacilli growth, and suppress coliform and staphylococci in rat faeces (Kusumawati *et al*., 2008). However, it is not known whether this strain presents an *in vivo* effect against *L. monocytogenes* ATCC 7644. In this present study, *L. plantarum* BSL was evaluated for antilisterial activity in rats using *L. monocytogenes* ATCC 7644 as indicator bacteria.

MATERIALS AND METHODS

Rats and diets

Sprague-Dawley rats (*Rattus norvegicus*) were obtained from Faculty of Animal Science, Bogor Agricultural University. A total of 60 rats (5-6 weeks of age) 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. 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 corn starch.

Bacterial culture

Seven LAB isolates (*L. plantarum* BSL, *L. plantarum* kik, *L. rhamnosus* R23, *L. plantarum* pi28a, *L. plantarum* ip, *L. plantarum* tpyk, *L. plantarum* MB427) were obtained from Laboratory of Food Microbiology, Department of Food Science and Technology, Bogor Agricultural University. These LAB strains were 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. *Lactobacillus monocytogenes* strain ATCC 7644 was cultured in 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.

To prepare cell suspension, a 24 h LAB culture 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 LAB cells in suspension was enumerated in MRS agar. Similar method was applied for *L. monocytogenes* ATCC 7644, only the media used was BHI broth.

Determination of antimicrobial activity of *Lactobacillus* sp against *L. monocytogenes* ATCC 7644 by *in vitro* assay (Nuraida *et al*., 2012)

Antibacterial activity of seven *Lactobacillus* sp isolates mentioned above were determined against *L. monocytogenes* ATCC 7644 using direct contact method. The culture of all *Lactobacillus* sp. and *L. monocytogenes* ATCC 7644 were refreshed in MRSB and BHI media respectively, and incubated for 24 h at 37 °C and 35 °C, respectively. One mL suspension of *Lactobacillus* sp (10^8 CFU/mL) and *L. monocytogenes* (10^5 CFU/mL) from appropriate dilution were further inoculated into tube containing 8 mL of skim milk and incubated at 35 °C for 24 h. The population of *L. monocytogenes* before and after incubation were counted on Listeria Selective Media Agar. *Lactobacillus* isolate that showed the highest antimicrobial activity was selected for *in vivo* experiment.

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

Rats having initial weight of 100-140 g were acclimatized for 7 days 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 for control group).

LAB strain which showed high activity against *L. monocytogenes* ATCC 7644 obtained from *in vitro* assay was used in this *in vivo* assay. The selected LAB strain and *L. monocytogenes* ATCC 7644 were grown in MRS broth and BHI broth respectively for 24 h at 37 °C. These cultures 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 culture (10^9 CFU/mL/rat) at day-3 for both treatment and control groups.

Rats were weighed every two days while feed consumption or feed efficiency was calculated daily. Rats were sacrificed by cervical dislocation in day-2 (before infected by *L. monocytogenes*), day-5, day-7, and day-9 (after infected by *L. monocytogenes*) to obtain samples for enumeration of LAB and *L. monocytogenes* population in faeces, caecum, and caecum content of rats. Analysis of IgA was carried out in serum samples taken from rat's heart before sacrificed using turbidimetric immunoassay method of Apo B Kit (KBC, 2009). For histopathology analysis, rat spleen and liver were stained using Hematoxylin-Eosin (HE) and observed the cell damage under light microscope (Kiernan, 1999). Data were evaluated using analysis of variance (ANOVA), and the

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significance of the differences was verified using Duncan test in SAS software (version 16).

RESULTS AND DISCUSSION

In vitro assay on antimicrobial activity of LAB against *L. monocytogenes* ATCC 7644

In general, all of the LAB strains tested showed antimicrobial activities against *L. monocytogenes* ATCC 7644 with different degree of inhibition at the range of reduction between 0.7–3.0 log CFU/mL. Two strains (*L. plantarum* tpyk and *L. plantarum* BSL) exhibited highest inhibitory effect (3 log CFU/mL) among other LAB strains (Figure 1). Wilson *et al*. (2005) found that *L. plantarum* SK1 displayed antimicrobial activity against *L. monocytogenes* UMCC98 by a reduction of more than 3 log CFU/mL. Aquilar *et al*. (2011) also reported that *L. plantarum* LB279 effectively inhibited the growth of *L. monocytogenes* CECT 4032 by 5 log unit compared to other pathogens.

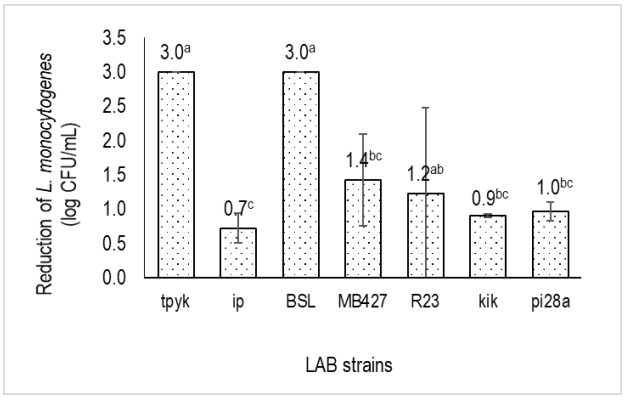

Figure 1: Antimicrobial activities of *L. plantarum* strains (tpyk, ip, BSL, MB427, kik, pi28a) and *L. rhamnosus* R23 against *L. monocytogenes* ATCC 7644. (All data were an average of two replicates, different letter superscripts following the values mean significantly different (p<0.05)).

Other species of *Lactobacillus* also exhibited antimicrobial activities. *L. salivarius* CECT5713 displayed antimicrobial activity against *Salmonella choleraesuis* CECT4155 (Olivares *et al*., 2006). *Lactobacillus rhamnosus* R14, *L. rhamnosus* R23 and *L. rhamnosus* B16 demonstrated strong antimicrobial properties against *E. coli* K1.1 (Nuraida *et al*. 2012). In addition, *Lactococcus piscium* CNCM I-4031 was able to reduce the population of *L. monocytogenes* by 3–4 log during 24 h (Saraou *et al*., 2016). In general, probiotics promoted desirable effects on health, but the positive effects of probiotics were usually attributed to strain specific (Williams, 2010). Based on the result of *in vitro* analysis, *L. plantarum* BSL was selected to be evaluated further for antilisterial activity in rats challenged with *L. monocytogenes* ATCC 7644.

Body weight gain and feed consumption of rats

Significant changes were observed during the experiment including weight gain and feed consumption of rats (Table 1). Both control and experiment group showed weight gain at day 2, but significantly higher value (p<0.05) was observed in experiment group than control group. The results showed that administration of *L. plantaru*m BSL for 2 days in the treatment group was able to increase the weight gain of rats. Further administration of *L. plantaru*m BSL did not affect the weight gain of rats, no further additional weight gain of rats was observed until 9 day-experiment either in control or treatment group.

The average feed consumption of 9-day-treatment in the treatment group was significantly higher (12.1 g/rat/day) than the control group (10.8 g/rat/day) (Table 1). This indicated that the administration of *L. plantarum* BSL could improve feed consumption of rats infected by *L. monocytogenes*. Similar result was found by Oyetayo (2004) that *L. acidophilus* was able to increase feed consumption and body weight gain of rats infected by enterotoxigenic *E. coli* (ETEC). Gross *et al*. (2008) also reported that *L. plantarum* 299v was able to increase feed consumption and body weight gain in diarrhea rats. In addition, *L. plantarum* 2C12 and *L. acidophilus* 2B4 isolated from Indonesian meat were also able to improve feed consumption and body weight gain of rats infected by enteropathogenic *E. coli* (EPEC) (Arief *et al*., 2010).

The presence of probiotic including *L. plantarum* BSL possibly increase the absorption of nutrients by producing some digestive enzymes, such as proteolytic enzymes. In addition, probiotic also is able to release of free amino acid and synthesis of vitamins that are needed for the growth of host (Parvez *et al*., 2006).

Table 1: The effects of orally administered *L. plantarum* BSL on feed consumption and body weight gain of rats during nine-day experiment. Different superscripts following the values in the same column and row mean significantly different (p<0.05).

group	Rats weight gain (g/rat)				Feed consumption (g/rat/day)
	Day-				
	2	5	7	9	
	(Before infected)		(After infected)		
Control	3.83^{b} ±0.23	4.17^{b} ±0.49	4.17^{b} ±0.23	5.50^{b} ±1.17	10.78^{b} ±2.15
Treatment	10.35^{a} ±2.35	9.84^{a} ±1.64	7.00^{a} ±1.41	7.33^{a} ±1.41	12.06^{a} ±1.27

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Table 2: The effects of orally administered *L. plantarum* BSL on total LAB population in the faeces, caecum, and caecum content of rats, either on both control and treatment group during nine-day experiment. Different superscripts following the values in the same column and row mean significantly different (p<0.05).

Group	Day of treatment			
	2 (Before infected)	5	7 (After infected)	9
Faeces (log CFU/g)				
Control	8.0±0.02^{b}	7.9±0.37^{b}	7.7±0.61^{b}	8.0±0.39^{b}
Treatment	9.1±0.00^{a}	9.4±0.00^{a}	9.3±0.18^{a}	9.4±0.01^{a}
Caecum (log CFU/cm^2)				
Control	7.7±0.30^{c}	7.7±0.35^{c}	7.8±0.26^{bc}	7.8±0.61^{abc}
Treatment	8.9±0.45^{a}	8.9±0.59^{ab}	9.0±0.51^{a}	8.7±0.45^{abc}
Caecum content (log CFU/g)				
Control	7.8±0.27^{b}	7.5±0.18^{b}	7.8±0.30^{b}	8.1±0.37^{b}
Treatment	9.1±0.16^{a}	9.0±0.49^{a}	9.4±0.03^{a}	9.2±0.27^{a}

Total LAB in caecum

LAB integrity in the gut was a fundamental factor for probiotics (FAO/WHO, 2002b). The number of LAB in caecal mucosa demonstrated that LAB attached on the mucosa of caecum (Table 2). In rats, administration of *L. plantarum* BSL could affect total LAB in the caecal mucosa. At day-2, administration of *L. plantarum* BSL increased total LAB in the caecal mucosa compared to control. The results revealed that in day-2 (before infected by *L. monocytogenes*). total LAB in the caecal mucosa of the treatment group was 8.9 log CFU/cm^2 which was approximately 1.2 log CFU/cm^2 higher than the control group (7.7 log CFU/cm^2). In day-5, day-7, and day-9 (after infected by *L. monocytogenes*), the number of LAB were not significantly different in both control (7.7–7.8 log CFU/cm^2) and treatment group (8.7–9.0 log CFU/cm^2).

Table 2 presented higher total LAB in the caecal mucosa of treatment rats than control, indicating that *L. plantarum* BSL was able to get through barriers in digestive tract including low pH (in the stomach), bile salt (in the gut), and ultimately reached caecal mucosa in the small intestine. Kusumawati *et al*. (2003) reported that *L. plantarum* BSL was resistant to 5% bile salt and pH 2.5, suggesting that *L. plantarum* BSL had high survival and ability to adhere to the epithelial cell surface. Gross *et al*. (2008) found that *Lactobacillus spp*. population in the small intestine of rats treated with *L. plantarum* 299v was higher (10^6 CFU/g) than control (10^5 CFU/g). Emmawati *et al*. (2016) stated that *L. plantarum* MB427 had adhesion and competition ability with *L. monocytogenes*, EPEC and *S. Typhimurium*, thus enabling the LAB to attach on the intestinal mucosa.

Total LAB in caecum content

Microflora composition in the caecum content may represent microorganism contain in the faeces. Rats caecum was a site in which some food nutrients were fermented by gut microflora as observed in human colon (Liong and Shah, 2006).

In the treatment group, administration of *L. plantarum* BSL significantly increased (p<0.05) total LAB in caecum content compared to control group (Table 2). At day-2 (before infected by *L. monocytogenes*), total LAB in the caecum content (9.1 log CFU/g) was higher than the control group (7.8 log CFU/g). At day-5, day-7, day-9 (after infected by *L. monocytogenes*), the number of LAB were not significantly different in both control (7.5 – 8.1 log CFU/g) and treatment group (9.0–9.4 log CFU/g).

Total LAB in the caecum content of the control group was lower than the treatment group. Similar findings were also found related to faeces and caecum of rats. *L. plantarum* BSL was able to increase total LAB in the caecum content by 1.4 log CFU/g. This result was in accordance with Arief *et al*. (2010) that probiotic treatment could raise LAB in the caecum content by 1.0 log CFU/g in comparison with control.

Lactobacillus plantarum BSL was prominently improved total LAB in the caecum content of rats treated with *L. monocytogenes*. *L. plantarum* BSL showed proper growth in digestive tracts, leading to higher total LAB in the caecum content. Emmawati *et al.* (2015) also reported that rats with the treatment of *L. plantarum* MB427 could get through the gastrointestinal tract of rats and retain their population in comparison with control.

Effects of *L. plantarum* BSL administration on the population of *L. monocytogenes* in faeces, caecum, and caecum content

Numbers of L. monocytogenes in faeces

Administration of *L. plantarum* BSL on rats enhanced the number of LAB population in faeces of rats, which was negatively correlated with *L. monocytogenes* population in faeces. At day-2 (before infected by *L. monocytogenes*), *L. monocytogenes* was found in the faeces (3–4 log CFU/g) in both control and treatment group (Table 3). The presence of *L. monocytogenes* in the faeces before rats infected by *L. monocytogenes* may be obtained from feed consumption and rice husk during adaptation for seven days.

At day -5, -7 and -9, after infected by *L. monocytogenes* the number of *L. monocytogenes* in the faeces in the treatment group (5.1–5.5 log CFU/g) was lower than the control group (7.1–7.2 log CFU/g). This results suggested that *L. plantarum* BSL had antilisterial

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Table 3: The effects of orally administered *L. plantarum* BSL on number of *L. monocytogenes* in the faeces, caecum, and caecum content of rats, either on both control and treatment group during nine-day experiment. Different superscripts following the values in the same column and row mean significantly different (p<0.05).

Group	Day of treatment			
	2 (Before infected)	5 (After infected)	7	9
Faeces (log CFU/g)				
Control	3.89±0.04^{d}	7.19±0.10^{a}	7.09±0.04^{a}	7.17±0.11^{a}
Treatment	3.74±0.25^{d}	5.53±0.04^{b}	5.34±0.06^{bc}	5.11±0.13^{c}
Caecum (log CFU/cm^2)				
Control	3.91±0.01^{c}	7.07±0.16^{a}	6.80±0.48^{a}	6.89±0.55^{a}
Treatment	3.42±0.64^{c}	5.23±0.03^{b}	5.15±0.22^{b}	5.09±0.15^{b}
Caecum content (log CFU/g)				
Control	3.27±0.01^{c}	7.19±0.04^{a}	7.17±0.01^{a}	7.14±0.06^{a}
Treatment	3.07±0.12^{c}	5.53±0.08^{b}	5.47±0.47^{b}	5.32±0.25^{b}

activity in rats faeces upto 1.9 log CFU/g reduction compared to control.

The present result was in accordance with the result reported by de Waard *et al*. (2002) that administration of *L. casei* Shirota strain YIT9029 could improve resistance against *L. monocytogenes* strain L242/73 in rats. The occurrence of *L. casei* Shirota led to the reduction of *L. monocytogenes* in rat's faeces. However, contrast to the result reported by Bambirra *et al*. (2007) it was found that administration of *L. sakei* 2a did not affect the population of *L. monocytogenes* Scott A in mice faeces (*L. monocytogenes* population in both control and treatment group were similar to 10^9 CFU/g). This result suggested that reduction of *L. monocytogenes* depended on the species of LAB, as described by Williams (2010) that positive effects of probiotics were attributed to strain specific.

Numbers of L. monocytogenes in caecum and caecum content

Administration of *L. plantarum* BSL significantly (p<0.05) reduced *L. monocytogenes* population in caecum and caecum content of treatment group compared to control group (Table 3). Similar to faeces, *L. monocytogenes* reduction was also observed in caecum and caecum content. At day-2 the population of *L. monocytogenes* was 3–4 log CFU/g in both control and treatment group.

At day-5, day-7, and day-9, lower population of *L. monocytogenes* was observed in caecum (5.1–5.2 log CFU/cm^2) and caecum content (5.3–5.5 log CFU/g) of treatment group than caecum (6.8–7.1 log CFU/cm^2) and caecum content (7.1–7.2 log CFU/g) of control group. The number of *L. monocytogenes* at day- 5, -7 and -9 were relatively stable either in control or treatment group, assuming that there was no further growth of *L. monocytogenes*.

This result showed that *L. plantarum* BSL administration to rats significantly reduced the *L. monocytogenes* population in caecum and caecum content upto 1.8 log unit. LAB naturally occur in the digestive tract of the rat. Total LAB in caecum and caecum content of control and treatment group was about 7 log CFU/g and 9 log CFU/g respectively. The presence of LAB in caecum and caecum content significantly affected *L. monocytogenes* population. The results confirmed that *L. plantarum* BSL demonstrated antagonistic effects on *L. monocytogenes* in rats eventhough with lower activity than *in vitro* study. *In vitro* experiment showed higher reduction (3 log CFU/mL) of *L. monocytogenes* population by *L. plantarum* BSL Lower reduction of *L. monocytogenes* in rats might be due to challenging factors existed in gut environment related to the co-aggregation, competition and exclusion ability of *L. plantarum* BSL with the pathogen.

Lactobacillus monocytogenes could be inhibited by probiotics through the production of antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin. Reduction of pH as a result of organic acids (primarily lactic acid and acetic acid) produced in gut promoted bactericidal or bacteriostatic activity (Shah, 2007). de Waard *et al*. (2002) found that inclusion of *L. casei* Shirota strain YIT9029 could attenuate *L. monocytogenes* in rats caecum. Arief *et al*. (2010) reported that *L. acidophilus* 2B4 was effective in retarding *E. coli* growth in caecal mucosa and caecum content in comparison with control. In addition, *L. plantarum* MB427 was reported being able to compete and displace EPEC, thus decreasing *E. coli* population in caecum and caecum content of rat (Emmawati *et al*., 2015).

Effects of *L. plantarum* BSL on IgA response of rat serum

The immune response was evaluated using IgA antibody test. Administration of *L. plantarum* BSL showed that IgA of control (12.0–12.5 mg/dl) and treatment (11.5–13.5 mg/dl) group were not significantly different in both groups in day-2 (before infected by *L. monocytogenes*) and in day-5, day-7, day-9 (after infected by *L. monocytogenes*) (Figure 2). Administration of *L. plantarum* BSL after 9 days did not significantly affect the IgA responses. According to Ward (2012) *L. monocytogenes* ATCC 7644 strain used in the present study was known included in serotype 1/2c that recognized to have lower risk than another serotype (Jaradat and Bhunia, 2003). This may explain that this strain did not affect the IgA response.

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Ren *et al*. (2015) reported that *Lactobacilli* strains showed the capacity to induce IgA production differently but after 20 days of administration the level of IgA was relatively stable and showed no significant difference between control and treatment groups. However, Galdeano and Perdigón (2006) reported that IgA response increased after 7 days of *L. casei* CRL 431 administration compared to control. Several studies have demonstrated that ability of probiotics to enhance immune responses were strain specific (Amalaradjou and Bhunia, 2012).

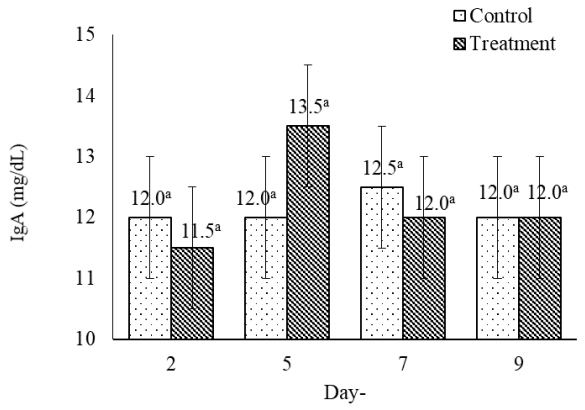

Figure 2: The effects of orally administered *L. plantarum* BSL on IgA response of rats during nine-day experiment. [All data were an average of two replicates, different letter superscripts following the values mean significantly different (p<0.05)].

Effects of *L. plantarum* BSL on damage of rat liver and spleen

The histopathology analysis was performed to observe the ability of *L. plantarum* BSL in retarding the damage of liver and spleen. The results revealed that administration of *L. plantarum* BSL showed by the descriptive analysis could reduce tissue damage of liver (Table 4) and spleen tissues (Table 5) compared to control. As shown in Table 4, before infected with *L. monocytogenes* (day-2), rat's liver tissues were free of vacuolization of hepatocytes cell but other forms of damages inlcuding edema, necrosis, infiltration of lymphoid cells, and proliferation of bile tract were observed, either in control or treatment group, with various levels of damages. Administration of *L. plantarum* BSL for 9 days after rats infected with *L. monocytogenes* showed evidence in reducing the damages observed mainly for edema, necrosis and infiltration of lymphoid cells of liver tissues. On the other hand, the damages of infected rats in control group were still observed and even more severe at day 9 with necrosis level increased to heavy level (+++) in 50% of the rats population (3/6). In contrast, treatment group at day-9 showed no necrosis in four rats (4/6) and minor necrosis in other rats (2/6). Edema, vacuolization of hepatocyte cells and proliferation of bile tract of infected rats (5/6) in control group were still observed until day-9-experiment and one rat (1/6) showed heavy (+++) level of bile tract proliferation. In the experiment group, negative vacuolization was detected in all rats (6/6), and minor level of edema and proliferation were detected in 50% population (3/6), while the rest of population (3/6) were negative.

Spleen damage in rats was observed inluding depletion of lymphoid cells, extension of pulp area and cell proliferation as shown in Table 5. Similar to liver damage, damages of rats spleen such as depletion of lymphoid cells and extension of pulp area but no cell proliferation were detected at day 2, before infected with *L. monocytogenes* either in control or experiment group. Administration of *L. plantarum* BSL for nine days could be able to reduce the spleen damage, as observed in treated rats, while in control rats, all forms of spleen damage were still observed at day 9. In the treated rats, 4 rats (4/6) were free of lymphoid depletion, and 2 rats (2/6) suffered only minor depletion, and no extension of pulp area were detected in most of the treated rats (5/6) and neither of cell proliferation occurred in all rats (6/6). As in control rats, most of them (5/6) were suffer of lymphoid depletion and extension of pulp area, but only two rats (2/6) showed minor proliferation. These results provided evidents that administration of *L. plantarum* BSL were advantage in reducing tissue damage of liver and spleen of rats. This indicates that the administration of *L. plantarum* BSL during nine-days was able to reduce the damage of liver and spleen tissue compared to control.

As previously reported by de Waard *et al*. (2002) that administration of *L. casei* Shirota strain YIT9029 on rats infected by *L. monocytogenes* L242/73 showed no significant difference between control and experimental groups as histopathological lesions due to *L. monocytogenes* infection were not obvious in the spleen and the same number of necrotic foci in liver after infected during 2-day. Martins *et al*. (2009) also reported that administration of probiotic bacteria did not cause an alteration in the morphology of intestines, liver, and spleen. In addition, the number of küpffer did not significantly different between control and experimental groups.

However, the results reported by Bambirra *et al*. (2007) showed the opposite way with those of the previous study, that the administration of *L. sakei* 2a could be able to reduce tissues damage of liver and spleen rats infected by *L. monocytogenes* Scott A compared to control. Shown by lesions were more severe in the control group and characterized by a destruction of the mucosa with infiltrate of inflammatory cells, predominantly macrophages and neutrophils. Moreover, dos Santos *et al*. (2011) also reported that administration of *L. delbrueckii* UFV-H2b20 significantly reduced the damage of liver caused by infection of *L. monocytogenes* 10403S.

Administration of *L. delbrueckii* UFV-H2b20 was significantly reduced the size of the inflammatory infiltrate, relative area of inflammatory lesions was smaller, tissue damage such as necrosis and degenerative changes of hepatocytes lower than the control (dos Santos *et al*., 2011).

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Table 4: Reduction of liver damage during nine-day administration of *L. plantarum* BSL.

Group	Number of rats	Days																			
		2					5					7					9				
		Liver																			
		Edema	Necrosis	Cell infiltration of lymphoid	Vac	Prolif	Edema	Necrosis	Cell infiltration of lymphoid	Vac	Prolif	Edema	Necrosis	Cell infiltration of lymphoid	Vac	Prolif	Edema	Necrosis	Cell infiltration of lymphoid	Vac	Prolif
Control	1	-	+	-	-	++	+	+	+	-	-	+	+	+	+++	-	+	+++	+	-	-
	2	+	++	-	-	+++	+	+++	-	+	++	+	++	-	-	++	+	+++	+	++	++
	3	+	+++	+	-	-	++	+	-	-	+	++	-	+	+++	+++	+	+++	-	-	+++
	4	++	-	-	-	+	+	+	+	-	+	+	++	-	-	+	-	+	-	-	+
	5	++	+	+++	-	+	+	++	++	-	+++	+	-	+	-	+	+	+	+	+++	+
	6	-	-	-	-	-	-	-	+++	-	+++	-	+++	+++	-	-	+	++	++	+	+
Treatment	1	-	+++	-	++	++	++	+++	-	++	++	+	+	-	+	-	+	-	-	-	-
	2	-	-	-	-	-	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-
	3	+	+	++	-	++	++	++	-	-	+++	-	+	++	+	++	-	-	-	-	-
	4	-	+	-	-	++	+	-	+	+	+++	-	+	+	-	+	-	+	+	-	++
	5	+	++	+	-	++	-	+	+++	-	+++	+	-	-	-	+	-	-	+	-	+
	6	-	-	-`	-	-	+	-	-	-	-	+	++	+	-	+++	+	+	+	-	+

Note: Damage level of liver tissue: no (-); minor (+); moderate (++); heavy (+++)
Prolif (proliferation of bile tract); Vac (vacuolization of hepatocytes cell)

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Table 5: Reduction of spleen damage during nine-day administration of *L. plantarum* BSL.

Group	Number of rats	Days											
		2			5			7			9		
		Spleen											
		Depletion of lymphoid cells	Extension of pulp area	Cell proliferation	Depletion of lymphoid cells	Extension of pulp area	Cell proliferation	Depletion of lymphoid cells	Extension of pulp area	Cell proliferation	Depletion of lymphoid cells	Extension of pulp area	Cell proliferation
Control	1	+	+	-	+	-	-	-	+	-	+	+	-
	2	-	+	-	+	+	+	+	++	+	++	-	+
	3	+	-	-	+	+	-	+	-	-	+	+	-
	4	+	+	-	+	+	-	+	++	-	-	+	-
	5	+	-	-	+	+	+	++	-	+	+	+	+
	6	++	-	-	-	+	+	+	+++	+	++	-	-
Treatment	1	+	-	-	++	+	-	+	+	-	-	+	-
	2	+	+	-	+	+	-	+	-	-	-	-	-
	3	-	-	-	+	+	-	+	+	-	-	-	-
	4	+	-	-	-	-	-	+	+	-	-	-	-
	5	+	-	-	+	+	+	-	-	-	+	-	-
	6	-	-	-	-	-	+	-	++	-	+	-	-

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

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These results suggested that not all of *Lactobacillus* sp can reduce the tissues damage on liver and spleen due to *L. monocytogenes* infection. Some of *Lactobacillus* species have been reported to reduce tissues damage such as liver and spleen. Possibly through the production of antagonistic compounds, inhibited *L. monocytogenes* adhesion to tissues, competition for nutrients, and modulating functions of the immune system (improves resistance to an infection of pathogens, increase the macrophages activity and antibody or cytokine production) (Bambirra *et al*., 2007).

CONCLUSIONS

Lactobacillus plantarum BSL was shown by *in vitro* assay had the highest antimicrobial activity among seven indigenous LAB isolates tested against *L. monocytogenes* ATCC 7644. This result was further supported by *in vivo* study using *L. monocytogenes* infected rats. Administration of *L. plantarum* BSL for 9 days could be able to enhance total LAB by 1.2 log unit in caecum and 1.4 log unit in faeces and caecum content of rats and showed inhibitory effects of *L. monocytogenes* by reducing the population upto 1.8 log unit (caecum and caecum content) and 1.9 log unit (faeces) compared to control group. Histopathology analysis also demonstrated that administration of *L. plantarum* BSL could be able to reduce all types of liver and spleen damage observed but no change was found for IgA response compared to control.

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