Viability and Survival of *Lactobacillus acidophilus* Soygurt in the Gastric of Rat

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Abstract. Due to harsh environment that must be passed in digestive system, one of important characteristic of lactic acid bacteria is acid tolerance which leads an ability to survive in the gastric. In the current study, rat gastric was used to evaluate a viability and a survival of *Lactobacillus acidophilus* in soybean based soygurt. Preliminary in vitro study suggested that the growth of *L. acidophilus* was pH dependent. The soygurt containing 17.8 log10 CFU/mL of *L. acidophilus* was administered orally to the rats per day. After 7 days consecutive administration, the viability of *L. acidophilus* in gastric juice of rats was assessed. The protein profile and the presence of lactic acid in the gastric juice were analyzed. The result showed that 9.2 log10 CFU/mL of *L. acidophilus* was survived to pass in gastric. The pH of the gastric juice was more acidic in the presence of lactic acid after soygurt administration compared to control. The protein profile of juice contained specific protein with molecular mass about 100, 50, 37 kDa and small peptide which probably secreted as bacterial responses against gastric environment. These findings suggested that *L. acidophilus* in soygurt is viable and survived in gastric environment and this soygurt is qualified as an alternative probiotic for human, especially with lactose intolerance.

Keywords: *Lactobacillus acidophilus* · Lactic acid · Soygurt

1 Introduction

Lactic acid bacteria possess a significant contribution in the production of various types of fermented products, such as yogurt or cheese. A very diverse lactic acid bacteria has been known to be very useful for making such products, such as from genus *Lactococcus*, *Bifidobacterium Streptococcus* and *Lactobacillus* [1]. Particularly, bacterium of genus *Lactobacillus* has been consumpting for years without any harmful effects. Implementations of these organisms are now broadening not only as food consumption, but also for the health and medicine application. It has been postulated that accumulation of lactic acid bacteria, as probiotics, in human intestine are strongly connected to health and longevity [2].

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A number of noteworthy publications reported that the existence of lactic acid bacteria in the intestinal microbiota reduce risk for illness development. Thus, one of important characteristics of probiotics is acid tolerance [3]. It reflects the ability of the bacteria to survive in the acidic conditions of intestine, particularly in gastric, where many bacteria cannot tolerate this stress. The vehicles to which lactic acid bacteria are delivered play an important role. Therefore, the development of probiotic vehicle is needed to ensure that lactic acid bacteria are viable, survive and able to overcome the physical and chemical barriers in gastrointestinal tract [4, 5].

Eventhough most commercial yogurts are from bovine milk, the demand for non-bovine source is growing, particularly because of the allergy cases to bovine milk proteins and the growing of vegetarian [6]. Soy-based yogurt could be an alternative to replace bovine milk because of their nutritional advantage, such as Fig hypolipidemic, anticholesterolemic, and antiatherogenic properties; and also reduce the risk of hormone-related disorders [7].

The acidic environment is extremely lethal to most bacteria, especially to bacteria non-resistant to acid [8]. *Lactobacillus acidophilus* isolated from mamalian intestinal tracts have been reported to survive gastrointestinal transit. So, this bacterium is widely used as probiotic. Moreover, extensive studies on the effects of *L. acidophilus* for boosting human health have been well documented [9]. The aim of this study was to investigate a viability and survival of *Lactobacillus acidophilus* in soybean based-yogurt (soygurt). The study was conducted by using rat gastric as a model. To observe bacterial response against acid condition in gastric, we analyze protein and lactic acid profile of gastric juice after oral administrasion with *L. acidophilus* soygurt.

2 Materials and Methods

2.1 Microorganism, Media and Growth Condition

LactobacilluS acidophilus ATCC 15078 obtained from American Type Culture Collection (Rockville, Md, USA) was maintained at Microbiology Laboratory, Faculty of Medicine, Jenderal Achmad Yani University. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany) in proanalysis grade, except if specifically mentioned. The bacterial cells were routinely grown for 48 h at 37 °C on selective MRS-A agar plates. Soybeans were obtained from Bromo, Indonesia.

2.2 Soymilk Preparation

Soybeans were cleaned by tap water, and then air dried for 15 h. After soaking for 8 h in sterile water, soybean skin was peeled. With some additional sterile water (soybean:water = 1:8, w/v), soybean was homogenized, and subsequently was boiled for 30 min. The debris were removed by centrifugation and followed by paper filtration. The soy milk was sterilized and finally stored for further soygurt preparation.

2.3 In Vitro Study of pH-Sensitivity of *Lactobacillus acidophilus*

The effect of acid level on *L. acidophilus* survival was tested in vitro by growing a culture of *L. acidophilus* in soy milk with various pH. The pH of soy milk between pH 2 to 7 were prepared by adjusting soy milk with 1 M acetic acid or 0.1 M NaOH. *L acidophilus* in soy milk was incubated at 37 °C for 48 h. Total plate count method using MRS-A agar as selective media was applied to determine a number of *L. acidophilus* in soy milk.

2.4 Soygurt Preparation

Lactobacillus acidophilus grown in 3% tryptone soya broth media was used as a culture starter. Sterile soy milk used as fermentation media was aseptically mixed with 1% of culture starter. The mixed soy milk was incubated at 37 °C for 24 h. Finally, soygurts were stored at 4 °C for 24 h.

2.5 Administration of Lactobacillus Acidophilus' Soygurt to Rat and Determination of Viability

Animal experimentation was approved by The Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University, Ministry of Research, Technology and Higher Education, Republic of Indonesia (No. 1172/UN6.C1.3.2/KEPK/PN/2016). The ability of *L. acidophilus* in soygurt to survive in gastric was investigated by analyzing recovery of *L. acidophilus* in gastric juice after oral administration of soygurt to rats for 7 days. Adult male Wistar rats (n = 18, approximately 8 weeks old), obtained from PT. Biofarma, Indonesia, were adapted for 7 days. During adaptation, the basic feed of pellet was nutritionally complete diet (PT. Biofarma, Indonesia). Water was provided ad libitum.

The rats were divided into 2 groups, control (n = 2) and treatment (n = 16). *L. acidophilus* soy yogurts were orally administered as suspension to treatment rats by a single dose 3.6 ml/ 200 g of body weight, with concentration 17.8 ± 1.8 $\log_{10}$ CFU/mL. The control rats were feed only with water and pellet without any soygurt administration. After consecutive 7-days administration, the rats were slaughtered. Then, the rat gastrics were immediately collected and were washed with phyisiological NaCl. The gastric juices were isolated by centrifugation at 3000 rpm for 10 min. Total plate count method was used to estimate the viability of survived *L. acidophilus* in rat gastric.

2.6 SDS PAGE and Lactic Acid Analysis

Protein profile of gastric juices of control and treated rats were compared. Samples were subjected to SDS PAGE (12.5% of separation gels and 4% of stacking gels) [10]. Protein bands were visualized by staining with Coomassie Briliant Blue R250. Lactic acid in gastric juice was analyzed by HPLC using acetonitrile as a mobil phase [11].

2.7 Statistical Analysis

To test the assumption of data normality of pH sensitivity on *L. acidophilus* growth, we use Shapiro-Wilk's test. Subsequently, One Way ANOVA was performed to analyze

variance between pH sensitivity. Post hoc Tukey's honestly significant differemce (HSD) was used to compare means of data. One sampe T-test was applied on microbiological data on survival and viability in gastric juice using the SPSS program. The level of significance was set at $p < 0.05$.

3 Results and Discussion

3.1 In Vitro Study of PH-Sensitivity of *L. acidophilus*

Figure 1 demonstrated an effect of pH on *L. acidophilus* growth. *L. acidophilus* counted by total plate count method for all trials, reflected a viability of bacterium after 48 h of fermentation. At the low pH, the growth of *L. acidphilus* was extremely low (2.62 $\log_{10}$ CFU/mL). By increased pH, the growth *L. acidophilus* was elevated.

The peak of bacterial growth was 15.86 log10 CFU/mL at pH 6.5. At pH > 6.5, the level of bacterial growth was reduced up to 40%. Statistical analysis showed that there was a significant difference of total colony between pH condition. Eventhough, L. acidophilus was able to grow from highly acidic to neutral pH. The results suggested that L. acidophilus growth was pH-dependent and was predicted to survive in gastric juice.

Gastrointestinal track of human consists of various condition of environment. To get to the suitable place in the intestine of gastrointestinal track and to confer a health benefit on the host, the viability and survival power of probiotic bacteria are important.

Soygurt is a type of yogurt using a soy milk as a prebiotic material. Noteworthy study had assessed the characteristic of L. bulgaricus and Streptococcus thermophilus in the soygurt. The conditions present in the stomach including ionic strength, enzyme activity (pepsin), and mechanical churning have been shown to have an impact on the viability of probiotics. Eventhough, the peak of growth of L. acidophilus was in neutral condition, L. acidophilus was still viable and survived at pH 2. This finding was in contradiction with previously reported by Hood and Zotola indicating that L. acidophilus in soy yogurt had a good viability and survival capability in acidic condition [12].

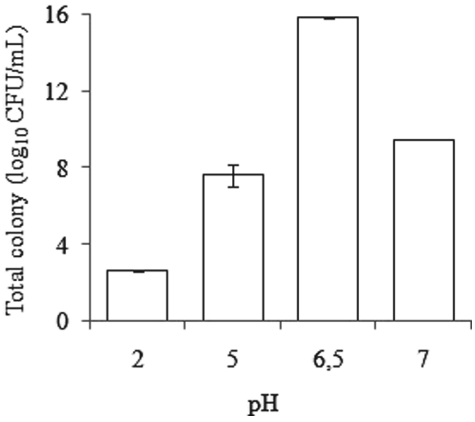

Fig. 1. Effect of pH on *L. acidophilus* growth.

Fig. 2. The comparison of survival *L. acidophilus* in gastric juice and initial *L. acidophilus* in soygurt.

In the present experimental study, we have demonstrated that *L. acidophilus* in the form of soygurt was still remain alive in the very acidic gastric juice of mamalian and potentially applied as a probiotic.

3.2 Viability and Survival of *L. acidophilus* in Gastric Juice of Rats

For evaluating *L. acidophilus* in soygurt as potential probiotics, viability and survival of *L. acidophilus* in gastric rat was performed. The comparison of survival *L. acidophilus* in gastric juice and initial *L. acidophilus* in soygurt was shown in Fig. 2. The average of soygurt administered to the 16 rats for 7 days was 17.8 ± 1.8 $\log_{10}$ CFU/mL. After 24h of last administration, the gastric juice of the rats were isolated and the viability of *L. acidophilus* was counted. *L. acidophilus* in gastric juice of rats was 9.22 ± 0.6 $\log_{10}$ CFU/mL. It was approximately 50% of initial concentration of soygurt. Because pH 4 of gastric juice (data not shown), prediction 50% of reduced viability of *L. acidophilus* was relevant with the viability of this bacterium in vitro (7.6 ± 0.6 $\log_{10}$ CFU/mL). In contrast, no *L. acidophilus* detected in gastric juice of control rats.

There were significant statisticaly difference of *T-test* between the viability of control, soygurt and gastric juice ($p < 0.05$). This result strongly suggested that *L. acidophilus* in soygurt is acid tolerance. Thus, the viability and survival of *L. acidophilus* in soygurt were qualified as potential probiotics.

3.3 SDS PAGE and Lactic Acid Profile

Soluble protein profile of gastric juice from soygurt treated- and control rats showed significant difference (Fig. 3). Intrestingly, the most abundance proteins migrated on SDS PAGE with molecular mass ~100 kDa (p100), ~50 kDa (p50), ~37 kDa (p37) and small peptides or proteins (<14 kDa) were strongly observed in gastric juice of treated rats, while in control rats were not observable.

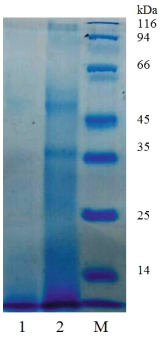

Fig. 3. Soluble protein profile of gastric juice from control rats (1) and soygurt treated rat (2).

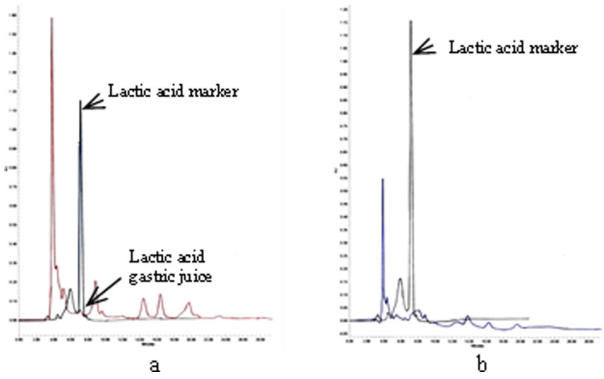

Fig. 4. The existence of lactic acid in the gastric juice was measured qualitatively from soygurt treated rat (b) and control rats (a).

The existence of lactic acid in the gastric juice was measured qualitatively (Fig. 4). The control rats showed no lactic acid in the gastric juice. In the soygurt treated rats, small amount of lactic acid was detected. This small amount was relevance to low viability and survival of *L. acidophilus* in gastric juice.

Furthermore, the viability and survival of L. acidophilus in soy yogurt were tested in vivo by using rats as animal model. Several studies, for instance, Curto and Ziarno have been conducted on viability and survival of probiotic bacteria through rats gastrointestinal track. All these studies demonstrated that probiotic bacteria has good viability and survival power to pass through the gastrointestinal track. Therefore, there was a significant correlation between our in vitro and in vivo studies [13, 14].

In highly acidic human intestine, lactic acid bacteria stress response can be reflected by their metabolic activities as well as its product, such as glucose or exopolysaccharides (EPS), specific peptides or proteins [15]. Gene expression of *L. acidophilus* that is responsible in acid tolerance was reported to express during the early log and stationarily phases [16]. In our study, we demonstrated and confirmed previous report that *L. acidophilus* in soygurt expressed three major high molecular and some low molecular weight proteins that were speculated to play a role in acid tolerance characteristic. The

lactic acid found in gastric juice indicated that *L. acidophilus* occupied lactic acid to help them to pass gastric as well.

It is plausible that a number of limitations could have influenced the results obtained from our study. We aware that our simulation of gastric juice using different pH solution did not completely mimic the true condition of gastric juice of rat or even human. Although performance was not ideal, we nevertheless believe that the outcomes will be closed to the situation in gastric of human.

4 Conclusion

To sum up, all results strongly confirmed and supported the survival and viability of *L. acidophilus* in soygurt in gastric juice. The upshot of this is the possibility of soygurt used as probiotic.

We expected that more detail study, particularly on proteomic analysis toward acid tolerance characteristics of *L. acidophilus* soygurt, enlighten our understanding on metabolic behavior of lactic acid bacteria in soy milk.

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