

RESISTANCE OF LACTIC ACID BACTERIA ISOLATED FROM INDONESIAN FERMENTED FOODS IN SIMULATED GASTRIC JUICE AND BILE SOLUTION

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ABSTRACT

The aim of this research was to study the viability of lactic acid bacteria isolated from Indonesian fermented foods in simulated gastric juice and bile solution. ten isolates i.e *L. plantarum* Mut 13, T3, Dad 13, Mut 7, *S. thermophilus* Mut 14, Mut 15, Mut 20, Mut 29, Dad 11 and *L. acidophilus* SNP2 were exposed to simulated gastric juice (pH 1,5, 2,0 and 3,0) and bile solution (1,5%, 2%, 3%). All isolated were tolerant to bile solution (1,5%, 2% and 3%) during 4 hours and 6 hours incubation. three isolates *S. thermophilus* Mut 29, Dad 11 and *L. plantarum* Mut 13 were more susceptible to 2% bile solution.

During exposure in simulated gastric juice, all isolates could not survive in simulated gastric juice pH 1,5 in 4 and 6 hours incubation. All isolates were tolerant in simulated gastric juice pH 2,0 during 4 hours of incubation and gave a lower population reduction i.e 3 log cycle up to 2 log cycle. After 6 hours of incubation six isolates (*L. sake* Mut 13, *S. thermophilus* Mut 14, Mut 15, Mut 20, Mut 29 and *Streptococcus* sp. Dad 11) exhibited a significant decrease 7 log cycle up to 5 log cycle. *Streptococcus* sp Dad 11 was more susceptible to simulated gastric juice pH 2,0 during exposure for 4 and 6 hours incubation. All isolates exhibit high resistance in simulated gastric juice pH 3,0 during 4 hours of incubation. During 6 hours of incubation, four isolates (*S. thermophilus* Mut 14, Mut 15, Mut 20 and Mut 29) decrease 5 log cycle. This research has shown that lactic acid bacteria isolated from Indonesian fermented foods has a potential to be a probiotic candidate (resistance in high pH and bile solution).

Keyword: Resistance, simulated gastric juice

INTRODUCTION

Indonesia has many kinds of fermented foods, which have been consumed for a long time. Indonesian fermented foods are fermented spontaneously by lactic acid bacteria and other microorganisms such as yeast and mould. Some species of lactic acid bacteria have been isolated and identified from Indonesian fermented foods such as *dadih*, *tempoyak*, salted cabbage, *gatot*, *growol* etc. some species of lactic acid bacteria isolated from Indonesian fermented foods are *L. plantarum* Mut 7, *L. sake* Mut 13 (Gatot), *L. casei subsp. rhamnosus* TGR 2 (growol), *L. plantarum* (tape singkong), *L. fermentum* (tempoyak) and *L. acidophilus* (salted young bamboo). A previous study (Rahayu *et al.*, 1996) reported that lactic acid bacteria isolated from Indonesian fermented foods has a potential to be a probiotic because they survive in acidic conditions, resistance to bile and has an antibacterial activity.

To be a probiotic organism, lactic acid bacteria isolated from fermented foods must be safe, viable and metabolically active within the gastrointestinal tract. Therefore, ingested lactic

acid bacteria isolated from Indonesian fermented foods must survive transit through the gastric environment and reach the colon in large quantities to facilitate colonization and thus to exert a beneficial effect on host and a dose of at least 10^8 cells per day has been recommended (Lianet *et al.*, 2003 ; Klingsberg and Budde, 2006).

To achieve this colonization lactic acid bacteria must overcome biological barriers that include acid in the stomach and bile in the upper intestinal tract (Noriega *et al.*, 2004; Lankaputhra and Shah, 1995). During digestion, the time from entrance to release of food from the stomach is around 90 minutes at a pH as low as 1.5 or 2.0. further digestive processes have longer residence times. Davenport (1977) in Noriega *et al.*, (2004) reported that bile concentration ranged from 1.5% to 2% in the first hour of digestion, and the levels decreased afterwards to around 0.3%. Thus, strains selected for use as probiotic bacteria should be able to tolerate acid for at least 90 min, tolerate bile acids, attach to epithelium and grow in the lower intestinal tract before they can start providing any health benefits.

Therefore, the aim of this research was to study the viability of ten isolated lactic acid bacteria isolated from Indonesian fermented foods in simulated gastric condition and also the resistance in bile solution.

MATERIAL AND METHOD

Bacterial strains and culture conditions

Ten lactic acid bacterias were purchased from *Pusat Studi Pangan dan Gizi* Gadjah Mada University. 2 isolates from *gatot* (*L. plantarum* Mut 7, *L. sake* Mut 13), *growol* (*L. plantarum* T3), 2 isolate from *tape ubi* (*S. thermophilus* Mut 20 and Mut 29), *ragi tape* 1 isolate (*S. thermophilus* Mut 14), *ragitempe* 1 isolate (*S. thermophilus* Mut 15), *dadih* 2 isolate (*Streptococcus* sp Dad 11 and *L. plantarum* Dad 13) and *L. acidophilus* SNP 2 (probiotic references isolated from infant feces).

After two successive transfers of the test organism in MRS broth (Oxoid) at 37°C for 20 h, the activated culture was again inoculated into MRS broth and incubated at 37°C for 20 h. It was then diluted with NaCl 0.85% and served as free cells in the survival studies.

Preparation of simulated gastric juice and bile solution

The simulated gastric juice was prepared by suspending pepsin (3 g/l) in saline (0,5%, v/v) and adjusting the pH to 1.5, 2.0, 3.0 with 1 M HCl and 1 M NaOH. To prepare bile solution, a solution of bile salts (Oxoid) was first prepared by dissolving amount (w/v) of bile salts in distilled water. This solution was then used to prepare 1.5%, 2.0% and 3.0% concentrations of bile. All solutions were sterilized at 121°C for 15 min.

Survival of local LAB in simulated gastric juice and bile solutions

Methods described by Lianet *al.*, (2003) were employed to compare the gastric and acidic resistance of various free cells of local LAB. Essentially , 1 ml of the free cells was added to 9 ml of simulated gastric juice (pH 1.5, 2.0, 3.0) and bile solutions (1.5%, 2%, 3%) and was vortexed for 20 s for complete dispersion of the free cells. Samples (0 h) were taken immediately after mixing. The samples were then incubated at 37°C and the mixture were taken every 4 and 6 h. the incubation time is according to residence time of food in the stomach for 2 until 6 h (Gropper and Groff, 2001).

Enumeration of lactic acid bacteria

Samples 1 ml taken immediately after mixing the free cells with gastric juice or bile solutions. They were serially diluted with 0.85% NaCl and pour plated on MRS agar containing 1% CaCO₃. Colonies were counted after incubation at 37°C for 24 h.

RESULTS AND DISCUSSION

Resistance in simulated gastric juice

To be used ad dietary adjuncts, local LAB must survive through the gastric condition in the stomach for at least 2 h (Gropper and Groff, 2001). Most of lactic acid bacteria species are susceptible to acid conditions. Jacobsen(1999) observed that from forty seven lactic acid bacteria strains, only twenty nine strains survived from acidic condition (pH 2.5). From Fig 1 we can see that, all strains could not survive from exposure to simulated gastric juice pH 1.5 for 4 h of incubation.

Simulated gastric juice pH 2.0 (Fig 2) gives a reduction 2 log cycle up to 4 log cycle at 4 h of incubation from initial count. Only one strain *Streptococcus* Dad 11 gives areduction up to 6 log cycle from initial count (10^{10} - 10^4 CFU/ml). Six strains (Mut 13, 14, 15, 20, 29 and Dad 11) gives areduction 5 log cycle up to 7 log cycle after exposed to gastric juice for a period of 6 h. Four strains (Mut 7, Dad 13, T3 and SNP 2) decrease 3 up to 4 log cycle, however the reduction of four strains was not significant and the viable number cells at 10^7 CFU/ml as suggested by Klingsberg and Budde (2006), gives a beneficial effect in the colon.

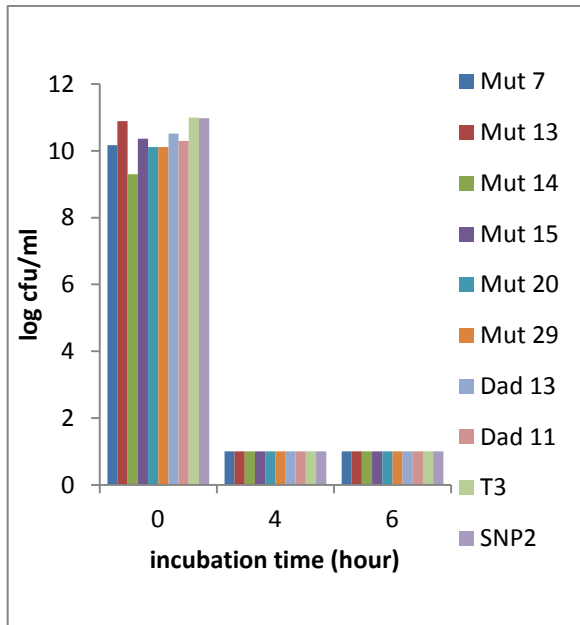


Fig.1

Fig 1. Resistance of local LAB to simulated gastric juice pH 1.5

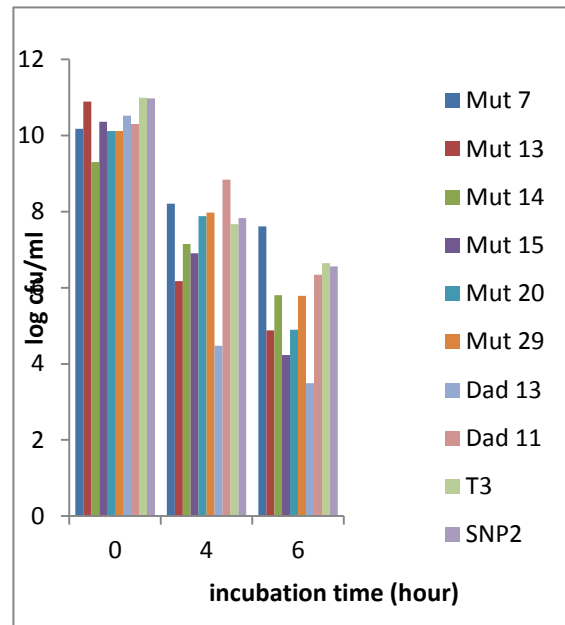


Fig. 2

Fig 2. Resistance of LAB in simulated gastric juice pH 2.0

All strains were quite resistant when exposed to simulated gastric juice pH 3.0 (Fig 3) at 4 h of incubation. The incubation time was prolonged until 6 h and four (Mut 14, 15, 20, 29) strains gave a significant reduction 5 log cycle from initial count. Two strains (Mut 7 and Dad 13) only decreased 2 log cycle at 6 h of incubation. Two LAB strain *L. plantarum* Mut 7 and *L. plantarum* Dad 13 has a potential to be a probiotic candidate, because of the ability to survive in acidic conditions (pH 2.0 and pH 3.0) for 6 h of exposure.

According to Annan *et al.*, (2008) using *in vitro* method to determine the viability of lactic acid bacteria gives a higher prediction of viable cells. The result of *in vitro* method cannot use to compare the result from *in vivo* method. The presence of food component can also raise the value of pH in the stomach. Food that contained carbohydrate gave extra energy to the cells to overcome the stress due to acid by using the Fo F1 ATPase. The Fo F1-ATPase is a known mechanism that gram positive organisms use for protection against acidic conditions. The role of the F0F1-ATPase in organism is devoid of a respiratory chain is to generate a proton motive force, via proton expulsion. As a consequence, it is thought that the F0F1-ATPase can increase the intracellular pH at a low extracellular pH (Corcoran *et al.*, 2005 and Sanz, 2007).

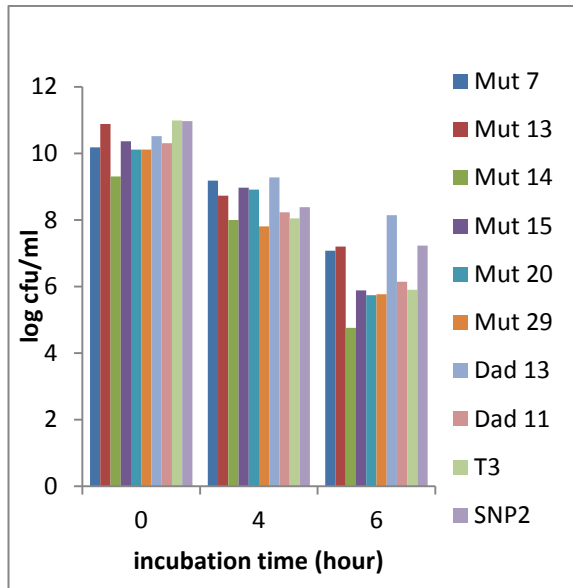


Fig 3.

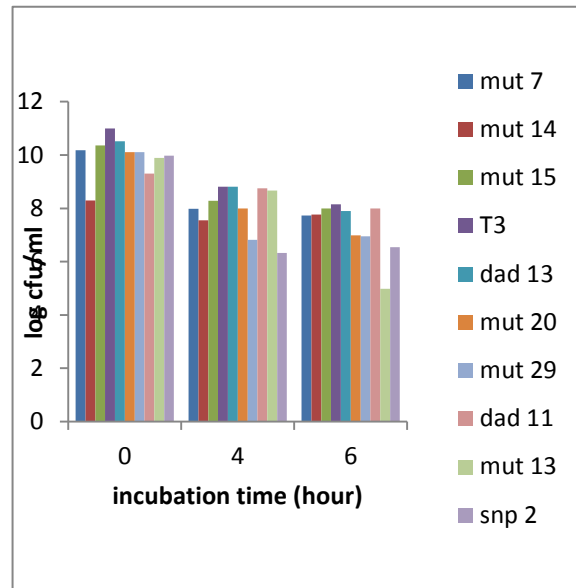


Fig.4.

Fig 3. Resistance of LAB in simulated gastric juice pH 3.0.

Fig 4. Resistance of local LAB to bile solution 1.5%.

In Figure 3 we can see that all local lactic acid bacteria exhibited different resistance to simulated gastric juice (pH 2.0 and pH 3.0) probably depended on the strain, source of LAB and the method we use. Acid condition is very harsh for the cells and inhibits the growth by denaturing the enzymes, damaging the lipopolysaccharide and external membrane of the cells. The reduction of pH in the cytoplasm can increase the permeability of the membrane cells, thus protons enter the cells in a large amount. If the number of protons inside the cytoplasm is greater than protons outside the cells or vice versa, thus it will inhibit or kill the cells. This is because cells do not have adequate energy to maintain the equilibrium of external and internal pH.

Resistance in bile solution

Being capable to survive bile concentrations produced in the human small intestines and to take up residence and multiply in human large intestine is another important characteristic of lactic acid bacteria to be used as probiotic dietary adjuncts (Gilliland, 1989). Tolerance of lactic acid bacteria to bile salts varied with species and strains, and different bile salts also influenced the resistance of lactic acid bacteria.

Survival of local lactic acid bacteria to bile solution (1.5%, 2% and 3%) after exposure for 4 and 6 h were shown in Fig 4, 5 and Fig 6. At bile solution 1.5%, all strains were more sensitive to bile 1.5% and 2% compared to bile solution at 3%. At bile solution 3% all strains were

decreased after 6 h of incubation. After 6 h exposed to bile, all strains decreased 1 log cycle up to 3 log cycle.

In the presence of bile solution 1.5% and 2% , three strains (Mut 7, Mut 29 and Dad 11) exhibited asignificant decrease after 6 h of exposure (Fig 4 and 5). Three strains decreased 4 log cycle up to 5 log cycle from initial count. On the contrary, all strains were more resistant to bile 3% after 6 h of incubation. As shown in Fig 6 all strains were not significantly decreased compared to bile 2%. All strains in 3% bile only decreased 1 log cycle up to 2 log cycle after 6 h of incubation. According to Antara *et al.*, (2009), lactic acid bacteria has a defense mechanism to overcome a biological barriers such as bile acids. Lactic acid bacteria produced bile salts hydrolase to minimize the toxicity of the bile. Bile salt hydrolase catalyses the hydrolysis of glycine or taurine-conjugated bile acids into amino acid residue and the bile acid. Strains with BSH activity come from intestinal environment in which they are exposed to bile salts. However, the fact that not all strains isolated from intestines have BSH shows that bacteria without this enzyme can either survive in this environment or survive the passage through this environment (Tanaka *et al.*, 1998). De smet *et al.*, (1995) suggest that BSH activity isa resistance mechanism against intracellular acidification by conjugated bile salts.

Bile acids are toxic to living cells and also increased the permeability of *L. acidophilus* membrane cells (Noh and Gilliland, 1993). Fatty acids can prevent membrane cells from leaking due to bile acids. Kimoto *et al.*, (2009) reported that supplementation of fatty acid C:18 in growth media can improve the resistance of lactic acid bacteria to bile acids. Kimoto *et al.*, (2009) also reported that, fatty acids maintain the stability of cells membrane and also fatty acids were occurring in the lipid membrane of gram positive bacteria. According to De smet *et al.*, (1995) the presence of carbon source i.e glucose or fructooligosaccharide in growth media reduces the toxicity of conjugated bile salts in *Lactobacillus* strains.

CONCLUSIONS

All isolated were tolerant to bile solution (1,5%, 2% and 3%) during 4 hours and 6 hours Incubation. three isolates *S. thermophilus* Mut 29, Dad 11 and *L. plantarum* Mut 13 was more susceptible to 2% bile solution. During exposure in simulated gastric juice, all isolates cannot survive in simulated gastric juice pH 1,5 in 4 and 6 hours incubation. All isolates were tolerant in simulated gastric juice pH 2,0 during 4 hours of incubation and give a lower population reduction i.e 3 log cycle up to 2 log cycle. After 6 h of incubation six isolates (*L. sake* Mut 13, *S. thermophilus* Mut 14, Mut 15, Mut 20, Mut 29 and *Streptococcus sp.* Dad 11) exhibit significant decrease 7 log cycle up to 5 log cycle..All isolates exhibit high resistance in simulated gastric

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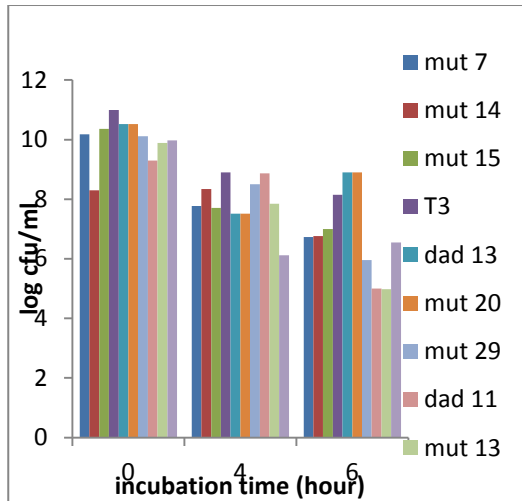


Fig.5

Fig 5. Resistance of LAB to bile 2%

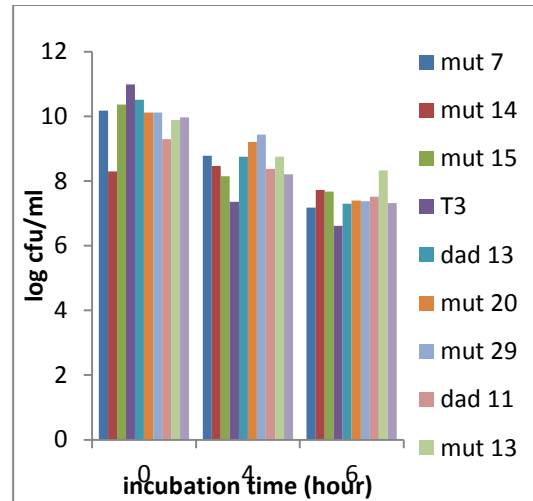


Fig.6.

Fig 6. Resistance of LAB to bile solutions 3%

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