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EVALUATION OF PROBIOTICS PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM BREAST MILK AND THEIR POTENCY AS STARTER CULTURE FOR YOGHURT FERMENTATION

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Abstract: *Purpose:* The objective of this study was to evaluate probiotic properties of four LAB isolated from breast milk, i.e. *Lactobacillus rhamnosus* R21, *L. rhamnosus* B16, *Pediococcus. pentosaceus* A16 and *L. fermentum* A17 and their potency as starter cultures for yoghurt fermentation.

Methodology: In vivo evaluation was done by feeding *Sprague Dawley* rats with standard diet and 10^9 cells of each LAB for 10 days.

Findings: The four isolates showed good survival both in pH 2 and bile salt for 5 h, and ability to suppress the growth of pathogenic bacteria. In vivo evaluation revealed that the four isolates reduced the number of *E. coli* in caecum, and increased the number of total LAB, without potency of invasion. They also induce proliferation of splenocytes, and A16 induce secretion of IgA in blood serum.

Value: R21 and B16 were potential as a single starter culture for yoghurt fermentation, while A16 have to be mixed with yoghurt starter cultures. The present finding suggests that the isolates are potential to be used for development of probiotic products.

Keywords: *Breast Milk; LAB; Probiotics; Proliferation of Splenocytes; Starter Culture*

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INTRODUCTION

Breast milk has been known to contribute to the colonization of lactobacilli and bifidobacteria in gastrointestinal track of new born baby (Martin et al., 2003, Ballongue, 2004, Diaz-Ropero et al., 2007, Olivares et al., 2006a). It is known that breast milk contains bifidigenic factor that stimulate the growth of bifidobacteria (Ballongue, 2004). The ability of lactobacilli and bifidobacteria to survive in and colonize the gastrointestinal track has been associated with various health-promoting properties (Ballongue, 2004, Mikelsaar et al., 2004). The colonization of those bacteria decreased with the increase of age of the host (Ballongue, 2004). In recent years there has been interest in incorporating those bacteria in live form (called probiotics) into food especially fermented milk to counteract harmful bacteria in the gastrointestinal track and to promote health effect (Schillinger et al., 2005, Tamime et al., 2007). Probiotic LAB strains have become important for research and commercial development in the area of food, nutrition and health (Ishida-Fujii et al., 2007).

Probiotic is defined as life microorganism when ingested in sufficient amount will confer beneficial effect to the host (FAO/WHO, 2002). Several criteria have to be met for selecting probiotic strains. Those include acid and bile tolerance, survival through the gastrointestinal track, ability to adhere to intestinal surfaces, exhibiting antimicrobial activity against potential pathogenic bacteria and good technological properties (Ouwehand et al., 2001). Other functional properties of probiotics include hypocholesterolemic activity by lowering plasma cholesterol (Liong and Shah, 2005, Pato et al., 2005), anti-infectious barrier to pathogens (Ballongue, 2004, Mikelsaar et al., 2004), preventing and treatment of diarrhoea (Rolfe, 2000, Salminen et al., 2004). and altering immune system (Nagao et al., 2000, Drakes et al, 2004, Diaz-Ropero et

al., 2007, Kotani et al., 2010). Probiotic properties and their functional properties are strain dependent and all probiotic strains are unique and different, therefore their properties and characteristics should be well defined (Salminen et al., 2004).

Human breast milk has been reported as source of lactic acid bacteria potential as probiotics. *B. longum* was the most widely found in breast milk, followed by B. *animalis, B. bifidum, B. catenulatum* (Gueimonde et al., 2007). Occurrence of lactobacilli in human breast milk were also reported such as *L. gasseri, L. fermentum, L. salivarius* (Martin et al., 2005). They showed that the lactobacilli isolates had potency as probiotic, at least similar to that of the strains commonly used in commercial probiotic products. *L. salivarius* CECT5713, *L. fermentum* CECT5716 (Diaz-Ropero et al., 2007), *L. gasseri* CECT5714 and *L. coryneformis* CECT 5711 (Olivares et al., 2006b) isolated from breast milk have also been reported to modulate immune respond. In a previous study, the authors isolated lactic acid bacteria (LAB) from breast milk of healthy lactating mother. The isolates consisted of *Lactobacillus, Pediococcus, Leuconoctoc, Streptococcus* and *Bifidobacteria* with the predominant isolates was *Lactobacillus* (Nuraida et al., 2007). To explore the potency of those lactic acid bacteria as probiotic candidate, four isolates, i.e. *L. rhamnosus* R21, *L. rhamnosus* B16, *P. pentosaceus* A16 and L. *fermentum* A17 were further characterised for their ability to survive in bile salt and low pH, to suppress pathogenic bacteria; and *in vivo* evaluation for ability to suppress *E. coli*, to increase LAB in caecum and colon, to stimulate proliferation of splenocytes and secretion of IgA; and potency of invasion. Among fermented milk products, the most important vehicle for the delivery probiotic microorganism is improtant (Tamime et al., 2007). Conventionally yoghurt is fermented with *Lactobacillus delbrueckii subsp bulgaricus* and

Streptococcus salivarius subsp thermophillus, which normally are unable to survive in gastrointestinal tracks (Agrawal, 2005). In contrast, the condition for yoghurt fermentation may not support the growth of probiotic bacteria. To obtain the desired health effect, probiotic organism should be present in a food to a minimum concentration of 10^6 cfu/g (Tamime et al., 2007). Therefore it is also important to evaluate the potency of probiotic candidates to be used as starter culture of yoghurt fermentation.

MATERIALS AND METHODS

Bacterial cultures

Four lactic acid bacteria (LAB) i.e. *L. rhamnosus* R21, *L. rhamnosus* B16, *L. fermentum* A17 *and Pediococcus pentosaceus* A16 previously isolated from breast milk were studied. Pathogenic bacteria used for evaluation of antimicrobial activity were *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus*. All cultures were obtained from SEAFAST Centre, Bogor Agricultural University. All cultures were maintained in stock culture kept in 20% glycerol at -20°C. When required the lactobacilli were grown in MRS broth while pathogenic bacteria in NB at 37°C.

Survival of lactic acid bacteria in pH2 and bile salt

LAB culture was grown once in MRSB from the stock freezer vial for 24 hours at 37°C before use in experiment. After this incubation, the LAB culture was inoculated into MRSB as control and MRSB acidified with concentrated HCl to pH 2, and incubated at 37°C for 5 hours. After incubation, plate counts were done using MRSA and the pour plate technique. The acid-tolerant strains were calculated by the difference between colony (log unit) grown on control and colony grown

on acidified MRSB (Modified from Chou and Weimer (1999) and Zavaglia et al., (1998))

Evaluation on survival on bile salt was done according to Ngatirah et al. (2000), but the bile salt concentration used was 0.5% (Moser and Savage, 2001). As much as 0.1ml LAB culture in 24 hours MRSB was added into 10 ml MRSB (control) and MRSB contained Oxgall salt 0.5%, and incubated at 37°C for 5 hours. The LAB was counted in MRSA. The survival was calculated by the differences of colony (log unit) grown on control and that grown on bile salt treatment.

Antimicrobial activity of the LAB isolates

Evaluation on antimicrobial activity of LAB isolates against pathogens was conducted using well diffusion agar method (Garriga et al., 1993). Each pathogenic bacteria culture was inoculated as much as 0.2 ml into Natrium Agar 100 ml, then about 20 ml NA contained pathogen culture was poured into the sterile plate and let until it solidify. The wells were made with diameter of 6 mm. As much as 30 µl of 24 hours LAB culture in MRSB was added into the wells, and incubated at 37°C for 2 days. The inhibition zone was defined as a clear area that formed around the wells.

Effect of orally administration of the lactic acid bacteria isolates on rats

Evaluation on the effect of LAB isolates on microbial count of caecum and colon of rats was done by orally administration of *Sprague Dawley* rats with the isolate for 10 days at the amount of 1 ml per day or equal to 10^9 cell. The LAB were grown in MRS agar for 48 h at 37°C. The colonies were harvested using cotton buds and suspended in 0.85% NaCl. Each group consisted of 6 rats. The rat was fed with standard diet throughout

the experiment (AOAC, 1984). The adaptation period prior to feeding with the isolate was 7 days, while administration of the lactic acid bacteria was done for 10 days. As a control, a group of rats administered with sterile water was used as a control. At the end of the experiment, the rats were terminated by cervical dislocation. The caecum, colon, spleen, kidney and liver were removed aseptically. The biomass of caecum and colon were analysed for total LAB (MRSA), total Lactobacilli (Rogosa Agar) and total *E. coli* (EMBA). The changes of total count, total LAB , *E. coli* and total lactobacilli were calculated by reducing the count obtained in treated rats with that of control. Kidney and liver were analysed for the presence of lactic acid bacteria to evaluate the invasion potency, while spleen was analysed for splenocytes. The spleen obtained above was washed with RPMI-1640 sterile. The splenocytes were counted using *Trypane Blue* method. Stimulation index was calculated by dividing the log value of splenocytes of treated rats with that of control. For the rats fed with *Pediococcus pentosaceus* A16, additional analyses was preformed for IgA and IgE of blood serum using ELISA kit done at a commercial laboratory.

Evaluation of potency lactic acid bacteria as starter culture for yoghurt fermentation

Evaluation of the isolates as starter culture for yoghurt fermentation was done as a single or combination with *Streptococcus salivarius subsp thermophillus.* The media for yoghurt fermentation with Lactobacillus was 12% skim milk, while that with *Pediococcus pentosaceus* and *L. fermentum* A17 was 12% skim milk supplemented with 3% sucrose. For evaluation of single culture, the media was inoculated with 2% of isolate previously grown in similar medium for 24 h. Incubation was done at 37 ^{o}C for 72 h. Analyses was done on pH, titratable acidity and total LAB every 24 h. For combination with *S.*

thermophillus, the ratio between LAB isolate and *S. thermophillus* were 1:1, 1:2 and 2:1 and incubated at 37°C and 42 °C for 48 h. Further evaluation was done using *P. pentosaceus* A16 or *L. rhamnosus* R21 in combination with *S. thermophillus* with the ratio 1:1. Incubation was done at 37°C and 42 °C for 48 h. Analyses was done on titratable acidity, pH and total lactic acid bacteria at 0, 6, 12, 24, 30, 36, and 48 h. Using ratio between lactic acid bacteria isolate and *S. thermophillus* 1:1, the rate of acid production and growth of lactic acid bacteria were evaluated during 48 h fermentation at 42°C. Titratable acidity was measured using titration method with 0.1N NaOH, while total lactic acid bacteria was enumerated on MRS Agar incubated at 37°C.

RESULTS AND DISCUSSION

Survival of lactic acid bacteria in pH2 and bile salt

The ability of LAB to survive on gastrointestinal tract was a requirement for bacteria to have a beneficial health effect after consumption (Martin et al., 2006). First stress on bacteria when they got into gastrointestinal tract was gastric acid exposure. Gropper et al. (2009) mentioned that pH of gastric acid is very low, i.e. about 2. In this experiment, the evaluation of acid resistance of LAB was conducted under low pH condition (pH 2) for 5 hours in accordance with the length of food reside in stomach (2-6 hours). *L. rhamnosus* R21, *L. rhamnosus* B16, *P. pentosaceus* A16 and *L. fermentum* A17 showed good survival on pH 2 and bile acid as shown by low reduction in their number (Figure 1). The most resistance to low pH and bile salts showed by *L. rhamnosus* B16. This finding confirms that resistance to low pH and bile salts are strain dependent. Schillinger et al. (2005) reported that strains of *L. acidophillus* isolated from probiotic dairy products were more tolerant to pH 2.0 that strains of

L. paracasei and *L. rhamnosus*. Tolerance to bile salt is considered to be an important character in strains envisaged as probiotics to grow and survive in the upper small intestine (De Smet et al. 1995). Several *Lactobacillus* had been reported to have bile salt hydrolase (BSH) activity to hydrolyze bile salt (De Smet et al. 1995). Of lactobacilli isolated from dairy products, BSH was detected in all strains of *L. acidophillus*, but not in strains of *L. casei* isolated form dairy products (Schillinger et al., 2005). The ability of the breast milk isolates to survive in low pH and bile salt indicates that these isolates could be considered as probiotic candidates.

Antimicrobial activity of lactic acid bacteria isolates

The antimicrobial activity of LAB was evaluated against four pathogenic bacteria, i.e. *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus*, and *Bacillus cereus* (Figure 2). All isolates showed quite good inhibition against *S. typhimurium*, with the highest inhibition showed by isolates A17 and B16. Isolates A16 and B16 show relative high inhibition activity (≥4 mm) against *E. coli*, while isolate A17 shows poor inhibition. Isolate R21 had a quite high inhibition activity against *B. cereus*. Isolate B16, A16 and A17 showed good inhibition against *S. aureus*, while the antimicrobial activity isolate R21 against *S. aureus* was to a lesser extent. The present results showed that antimicrobial activity against Gram positive bacteria was comparable with Gram negative bacteria, except *L. fermenteum* A17 which showed weak antimicrobial activity against *E. coli*. The present study also in agreement with previous study showing that antibacterial potency of lactobacilli varied among strains (Olivares et al. 2006a). Olivares et al. (2006a) examined antimicrobial activity of lactobacilli from human milk showing that four LAB isolates (*L. salivarius* CECT5713, *L. gasseri* CECT5714, *L. gasseri* CECT15, and

L. fermentum CECT5716) had antimicrobial activity against pathogen (*Salmonella choleraesuis)* with various degree. *L. salivarius* CECT 5713 was also reported to have antimicrobial activity against *Listeria monocytogenes* and *Klebsiella oxytoca* (Martin et al., 2006). *Lactobacillus casei I-5* isolated from an alcohol fermentation was reported to be able to prevent infection in mice due to *Escherichia coli* (Ishida-Fujii et al., 2007), while Lactobacillus GG was reported to prevent infection due to *Salmonella typhimurium* C5 (Hudault et al. 1997). The ability of the breast milk strains to inhibit pathogenic bacteria offers a protective effect againts infection when the isolate used as probiotic product.

There were some components generated by LAB with antimicrobial activity, i.e. organic acids, hydrogen peroxide, and protein or specific protein complex called bacteriocin (Ouwehand and Vesterlund, 2004). The acids caused pH reduction under pH of bacteria growth where these acids were in a dissociation form which can diffuse into bacterial cell. Some LAB also generates hydrogen peroxide in a considerable quantity. The accumulation of this component in LAB cell because it has no catalase (Ouwehand and Vesterlund, 2004).

Effect of orally administration of lactic acid bacteria isolates on microbial count of caecum and colon of rats

Table 1 shows changes in total count of caecum and colon biomass of rats after 10 days administration of LAB isolates as compared to control. Administration of *P. pentosaceus* A16 increased the total count of caecum biomass by almost 1 log as compared to control, however it was not observed in other isolates. Orally administration of *P. pediococcus* A16, *L. rhamnosus* R21 and *L. fermentum* A17 decreased the total

Isolate	Changes of Total count (cfu/g)		Changes in *E. coli* count (cfu/g)		Changes in Total LAB count (cfu/g)		Changes in Total Lactobacilli (cfu/g)	
	Caecum	Colon	Caecum	Colon	Caecum	Colon	Caecum	Colon
P. pentosaceus A16	0.98	-0.41	-1.03	-0.76	1.54	-0.03	NA	NA
*L. fermentum*A17	0.07	-0.65	-0.63	-0.85	1.15	-0.37	1.53	0.00
*L. rhamnosus*R21	-0.08	-0.33	-0.71	0.40	2.10	-0.45	1.10	-0.18
*L. rhamnosus*B16	-0.17	0.02	-1.53	0.68	1.41	-0.08	1.05	-0.21

NA=Not applicable

Table 1: Changes of Total Count of Caecum and Colon Biomass After 10 Days Orally Administration of Lactic Acid Bacteria Isolated from Breast Milk as Compared to Control

count of caecum biomass. While administration of *L. rhamnosus* B16 showed no significant effect on the caecum biomass (Table 1). All isolates reduced total *E. coli* in caecum, in contrast their increased the lactic acid bacteria count. Only *P. pentosaceus A16* and *L. fermentum* A17 also reduced *E. coli* in colon. In line with LAB count, total lactobacilli in caecum of rats orally administered with the isolates also increased. Feeding of isolates did not increase the LAB and lactobacilli in colon. This was in accordance with the number of *E.coli* in colon which is only slightly reduced by feeding of *P. pentosaceus* A16 and *L. fermentum* A17. Zanini et al., (2007) reported that consumption of yoghurt containing mixture of *L. casei* with other lactobacilli and bifidobacteria strains increased the lactobacilli by about 1 log cfu/g faeces in children after two weeks of consumption. The finding also suggested that the complex mixture of probiotic seemed to be more effective in enhance intestinal lactobacilli. The present research, feeding with LAB was done for only a single culture. Possibility to use the isolates in mix cultures should be considered as future development to amplify the

effectiveness of the isolates in enhancing intestinal lactobacilli. Analyses of kidney and liver for the presence of lactic acid bacteria revealed that no lactic acid bacteria were found in those organs showing that the isolates had no potency of invasion.

Effect of orally administration of lactic acid bacteria isolates on proliferation of splenocytes and stimulation of IgA

Figure 3 shows that administration of rats with LAB isolated from breast milk increased the splenochyte as compared to control. The highest stimulation index was observed in *P. pentosaceus* A16. The Lactobacilli isolates showed almost similar stimulation index on proliferation of splenocytes (Table 2). Translocation of whole probiotic bacteria or bacterial products over the epithelium may influence the gut and systemic immune system (Herias et al., 1999). Research by Amrouche et al. (2006) showed that splenocyte proliferation was stimulated to varying degrees by cytoplasm of *Bifidobacterium lactis* Bb12 with strong stimulation index i.e. 2.96 showed by crude cytoplasmic extract. They suggested that the mixture of macromolecules types may be more immunologically active.

Isolate	Stimulation index
P. pentosaceus A16	1.65
*L. fermentum*A17	1.48
*L. rhamnosus*R21	1.45
*L. rhamnosus*B16	1.49

Table 2: Stimulation Index of Lactic Acid Bacteria Isolated from Breast Milk on the Proliferation of Splenocytes

Lactobacillus paracasei strain NCC2461 has been reported to be able to induced IL-12 and IL 10, proliferation lymphocyte T-CD 41 and reduced IL-4, IL-5, IFN-γ (Von der Weid et al., 2001).

Further analyses on blood serum of rats fed with *P. pentosaceus* A16 revealed that this isolate stimulated the secretion of IgA in blood serum (Figure 4). The secretion of IgA in rats was affected by the dose of LAB cells administered to the rats, i.e. administration of the rats with 10^7 cfu has increased the IgA of blood serum by 2 folds as compared to the control, while that with 10^9 cfu has increased 2.67 folds. Secretion of IgA is the first line of defense which produce abundant mucosal antibodies mediating exclusion of foreign antigens by preventing epithelial adherence and penetration of invasive pathogenic microorganisms (Picard et al., 2005). *L. fermentum* CECT5716 isolated from human milk enhanced the production of Th1 cytokines by spleen cells and increased the IgA concentration in faeces from 87.2 mg/g in control to 107.9 mg/g in mice administered with the lactobacilli (Diaz-Ropero et al., 2007). Orally administration of mice with encapsulated *Bifidobacterium bifidum* enhanced IgA production in the culture of both mesenteric lymph nodes and spleen cells by 1.7 and 6.7 folds respectively (Park et al., 2002). Wungrath et al. (2009) reported that consumption of yoghurt containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* had increased IgA in blood serum of healthy adolescens, i,e, from 276 mg/dl to 312 mg/dl in male and 274 mg/dl to 309 mg/dl in female. Feeding 36 children with yoghurt containing *L. casei* and other strain of lactobacilli and bifidobacteria has increased secretion of IgA in saliva from about less than 20 mg/100 ml to about more than 20 mg/100 ml after 4 weeks of consumption (Zanini et al., 2007). Similar finding was also reported by Kotani et al., (2010) that salivary SIgA secretion rate in the elderly can

be increased from 319 μg/5 min to 359 μg/5 min by supplementation with LAB.

The present research indicate that administration of lactic acid bacteria isolates did not increase secretion of IgE as compered to the control that remained <1 IU/ml.

Evaluation of potency lactic acid bacteria as starter culture for yoghurt fermentation

Previous research revealed that the growth of *P. pentosaceus* in milk stimulated by supplementation of sucrose or glucose (Nuraida et al., 2011a). In this research media for milk fermentation using *P. pentsaceus* as single starter culture was skim milk supplemented with 3% sucrose. Use of lactic acid bacteria isolates a single starter culture revealed that isolate R21 and B16 could be used as a single starter culture as shown by tritratable acidity and drop in pH during fermentation (Figure 5). Although skim milk as fermentation medium has been supplemented with sucrose, however, *L. fermentum* was still unable to ferment milk as shown by low titratable acidity and pH of milk that was still above 5. The titratable acidity increased from 0.12-10.16% to 1.29-1.55% by lengthening fermentation time up to 72 h, with the highest titratabel acidity of 1.55% observed in *L. rhamnosus* B16. *P. pentosaceus* was able to acidify the yoghurt, although the titratable acidity (1.29%) was lower than that of *L. rhamnosus* R21 (1.37%) and B16 (1.55%), however the pH was almost similar i.e. 6-41-6.48 after 72 h (Figure 5).

In accordance with production of acid and lowering of pH, *L. rhamnosus* R21 and *P. pentosaceus* A16 reached 10^9 cfu/ml, with the highest count was found after 72 h fermentation (Figure 6). Lower lactic acid bacteria count found in milk fermented by *L. fermentum* A17 that only reached 10^8 cfu/ml.

The number of lactic acid bacteria in the present yoghurt met the suggested minimum number that should be present in product i.e. 10^6 cfu/g to give desired health effect (Tamime et al., 2007). The present results revealed that *L. fermentum* A17 was not suitable for milk fermentation as the isolate could not acidify the milk and their growth was also not as good as the other isolates. Sufficient acid was produced after 48 h fermentation and no significant difference between 48 h and 72 h.

Further experiment was to evaluate the combination between *L. rhamnosus* R21 or *P. pentosaceus* and *S. thermophillus* conventionally used as one of yoghurt starter cultures. The results revealed that combination of *P. pentosaceus* A16 with S. *thermophillus* decreased the pH and increased the total acid of final product better than single culture of *P. pentosaceus* A16 (Figure 7 and 8). Combination of *L. rhamnosus* R 21 with *S. thermophillus* did not affect the pH and total acid of yoghurt fermented at 42 ^{o}C, but it improved total acid at 37^{o}C. The ratio between LAB isolate and *S. thermophillus* did not affect the total acidity and pH of yoghurt after 48 h fermentation.

Using combination of lactic acid bacteria isolate and *S. thermophillus* with the ratio of 1:1, the rate of acid production and growth of lactic acid bacteria were further evaluated. The results revealed that acid production by *P. pentosaceus* as a single starter culture was lower than *S. thermophillus* or than combination of both (Figure 9). Combination of *P. pentosaceus* and *S. thermophillus* also improved acid production of a single cultures of *S. thermophillus*. It suggests that there was a mechanism of symbiotic between *P. pentosaceus* and *S. thermophillus* that should be further elaborated. The decrease in pH (data not shown) was also in accordance with the production of acid. In contrast to *L. rhamnosus* R21 that produced acid faster than *S. thermophillus* (Figure 9), combination of lactobacilli with *S. thermophillus* did not affect acid production. There was no significant different

in the total number of lactic acid bacteria between milk fermented by a single culture of *P. pentosaceus* A16 or *S. themophillus* and combination of the both lactic acid bacteria (Figure 9). Yoghurt fermented with *L. rhamnosus* R21 and with conbination with *S. thermophillus* contained slightly higher total lactic acid bacteria count that yoghurt fermented by *S. thermophillus* alone. Overall after 24 h fermentation, all yoghurt contained lactic acid bacteria of more than 10^8 cfu/ml which considerably high. Considerable number of lactic acid bacteria is suggested to compensate for possible losses during passage through the stomach and intestine (Tamime et al., 2007).

The present finding suggets that the lactic acid bacteria isolated from breast milk are potential for development of food product with specific health promoting effect. Information on the bacterial strains used in the product is important to met the requirement as probiotic product. The ability of the isolates to survive in low pH and bile salt, and to suppress pathogenic bacteria indicate that the isolate could contribute to the protective effect against infection by pathogenic bacteria. Research done by Hartanti (2010) using similar isolates showed that *L. rhamnosus* B16 reduced the number of rats suffering diarrhoea caused by EPEC K1.1. infection (2 of 6 rats as compared to 4 of 6 rats in control). Specific functional characteristic was also indicated by the isolates, i.e. their potency to alter immune respond, although this work is still preliminary. *P. pentosaceus* A16 used in the present research has also been reported to be able to assimilate cholesterol in considerable amount (14.03µg/ml) in the medium (Nuraida et al., 2011b). The food product as vehicle to deliver probiotic organism is also important. The present finding suggests that from the technological point of view, three lactic acid bacteria isolates (*L. rhamnosus* R21, B16 and *P. pentosaceus* A16), could be used as starter culture for yoghurt fermentation or mix with the conventional starter

cultures. Sensory characteristic of lactobacilli yoghurt was not significantly different with those fermented with conventional starter cultures (Nuraida et al., 2011a). Hence the present research has been part of the roadmap in developing of probiotic products using lactic acid bacteria of breast milk origin.

CONCLUSIONS

The four LAB isolated from breast milk were potential to be used as probiotics as shown by their ability to survive low pH and bile salt, and suppress pathogenic bacteria. The isolates had also potency to increase LAB count and suppress *E. coli* in caecum. No potency of invasion was observed in four isolates. The isolates also showed their potency as immunomodulator by stimulating proliferation of splenocytes. *P. pentosaceus* A16 was potential to induce secretion of IgA, but not induce secretion of IgE. The dose administered should be considered for the effectiveness of the effect. From the technology point of view, three isolates i.e. *Lactobacillus rhamnosus* R21, B16 and *P. pentosaceus* A16 could be used as starter for yoghurt fermentation. While *L. rhamnosus* R21 and B16 could be used as single starter culture for yoghurt fermentation, for better results *P. pentosacues* needed to be combined with *S. thermophillus*. The efficacy of the products is important to be evaluated in the future research.

BIOGRAPHY

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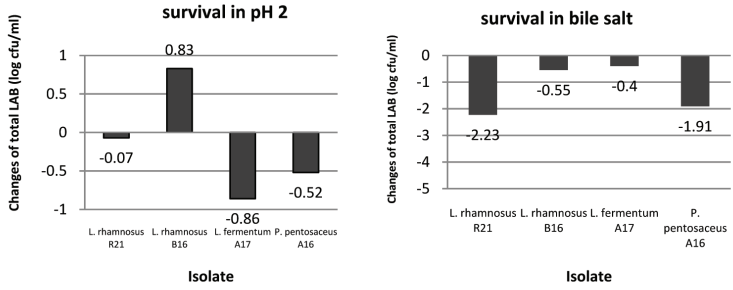

Figure 1:
Changes in Total Lactic Acid Bacteria Isolated from Breast Milk Prior to Exposure to pH 2 for 5 h and to 0.5% Bile Salt for 5 h at 37oC.

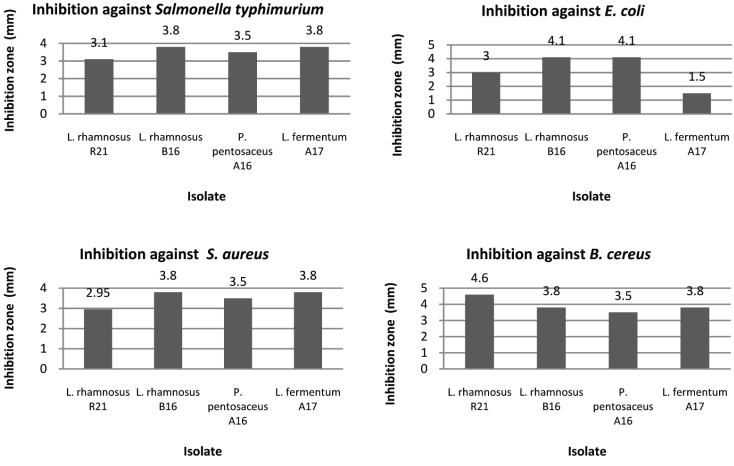

Figure 2:
Antimicrobial Activity of Lactic Acid Bacteria Isolated from Breast Milk against Pathogenic Bacteria

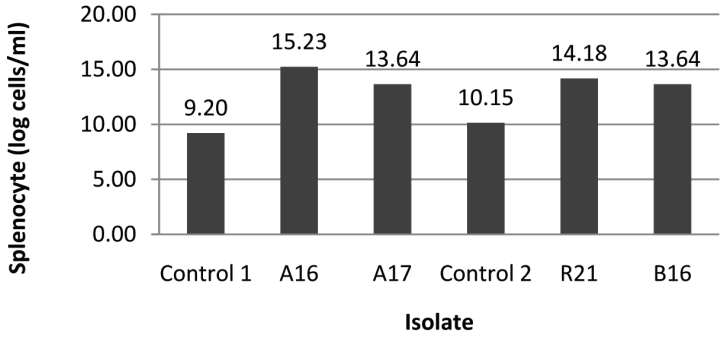

Figure 3:
Effect of Administration of Lactic Acid Bacteria Isolated from Breast Milk on Proliferation of Splenocytes of Rats.

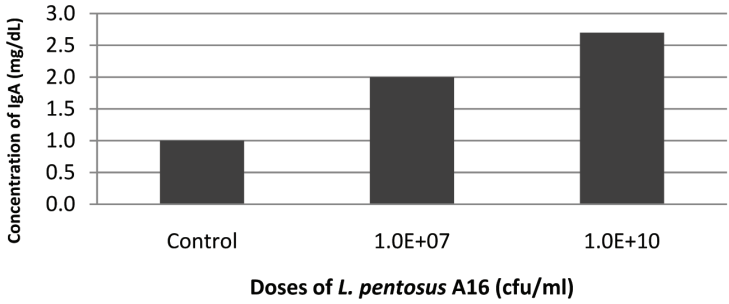

Figure 4:
Effect of Administration of P. Pentosaceus A16 Isolated from Breast Milk on the Secretion of Blood Serum IgA

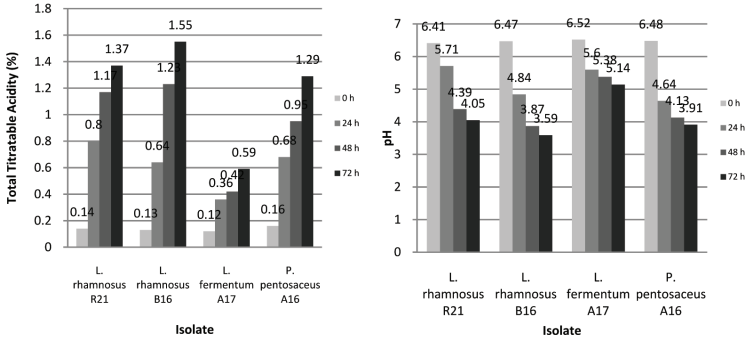

Figure 5:
Total Titratable Acidity and pH of Yoghurt Fermented by Lactic Acid Bacteria Isolated from Breast Milk as a Single Starter Culture.

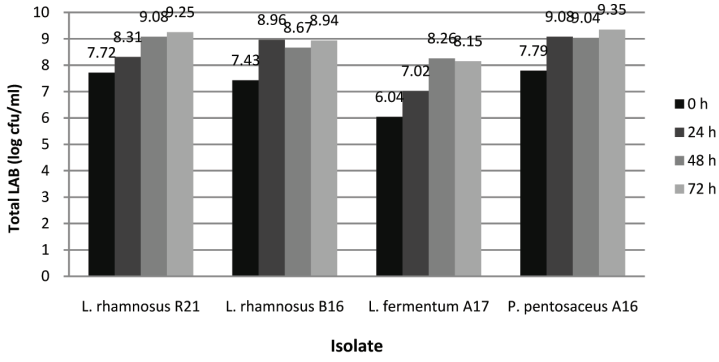

Figure 6:
Growth of Lactic Acid Bacteria During Yoghurt Fermentation by Lactic Acid Bacteria Isolated from Breast Milk.

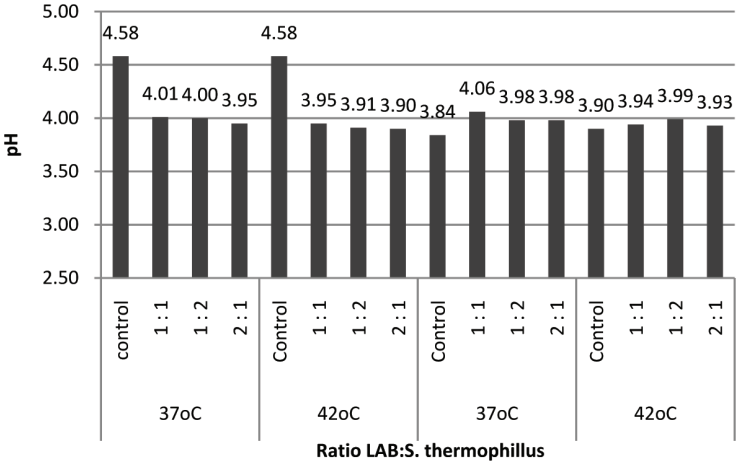

Figure 7:
pH Value of Yoghurt Fermented With Lactic Acid Bacteria Isolated from Breast Milk in Combination With S. Thermophillus After 48 h. Control was Yoghurt Fermented With a Single Culture of LAB Isolate.

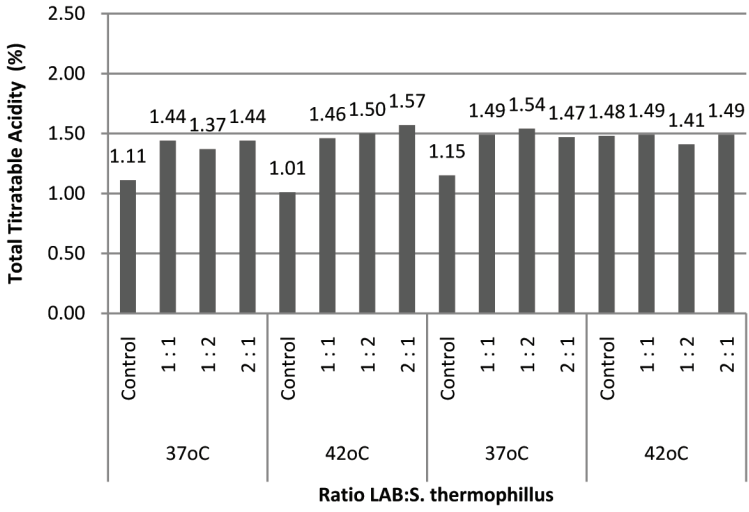

Figure 8:
Total Titratable Acidity of Yoghurt Fermented With Lactic Acid Bacteria Isolated from Breast Milk in Combination With S. thermophillus After 48 h.

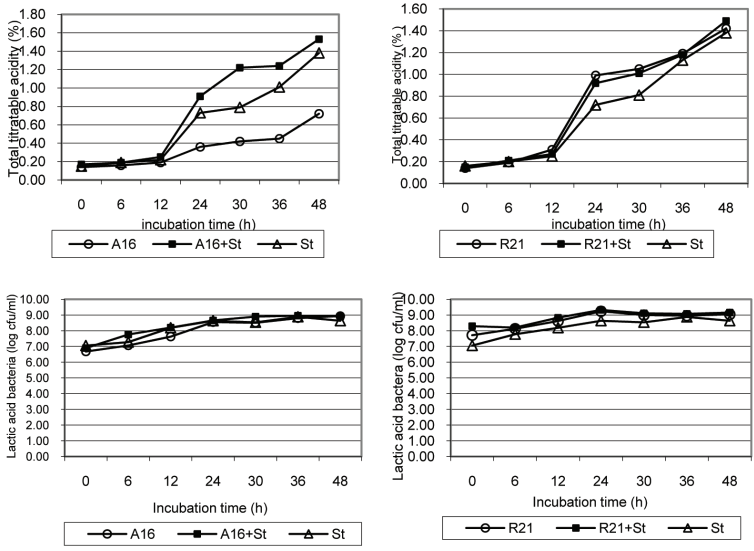

Figure 9:
Acid Production and Growth of Lactic Acid Bacteria Isolated from Breast Milk as a Single Culture and in Combination with S. thermophillus With the Ratio of 1:1, Incubated at 42°C.