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Novel probiotic *Enterococcus faecium* IS-27526 supplementation increased total salivary sIgA level and bodyweight of pre-school children: A pilot study

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ABSTRACT

Enterococcus faecium IS-27526 is a novel probiotic isolated from dadih, an Indonesian traditional fermented buffalo milk. A 90 days randomized double-blind placebo-controlled study ofpre-post trial was conducted in pre-school children with two groups, placebo and probiotic group. Ultra High Temperature low fat milk was used as a carrier in each group. The aims of this study were to investigate the effect of *E. faecium* IS-27526 in milk on humoral immune response and on bodyweight of pre-school children. Total serum IgA and total salivary sIgA were measured by sandwich ELISA. The bodyweight of young children was measured. The results showed that total serum IgA did not significantly increase in the probiotic group compared with the placebo group. Total salivary sIgA level and the bodyweight significantly increased ($p < 0.05$) in probiotic groups compared to the placebo. Changes of total salivary sIgA level were significantly higher in underweight children supplemented with probiotic. Weight gain was observed significantly in children with normal bodyweight supplemented with probiotic. Neither mortality nor weight loss was recorded throughout the study. Taken together, novel probiotic *E. faecium* IS-27526 has significant positive effects on humoral immune response, salivary sIgA, in underweight pre-school children, and on weight gain of pre-school children.

1. Introduction

The human intestinal tract harbors a large, active, and complex community of microbiota. The intestine is a highly sophisticated organ contains up to 400-500 different species of bacteria, both harmful and commensals [1]. This microbiota plays a key role in the host's overall health through its metabolic activities and physiological regulation such as promotion of nutrient absorption, synthesis of bioactive compounds, improvement of intestinal barrier function, motility, resistance to pathogens or modulation of the immune system [2]. Alteration of the microbiota may cause some direct or indirect digestive pathologies like infectious diseases, chronic inflammation [3] and metabolic disorders [4]. A healthy intestinal microbiota is considered to be important for priming of the infants' mucosal and systemic immunity.

The intestinal microbiota may be modified temporarily by nutritional changes in the diet. In particulary the consumption of pro- or prebiotics can restore or maintain the intestinal ecosystem by [5,6].

Probiotic defined as a "live microorganism which when administered in adequate amounts confers a health benefit on the host" [7]. Criteria for the selection of probiotics are safety, tolerance to gastrointestinal conditions, ability to adhere to the gastrointestinal mucosa and competitive exclusion of pathogens [8,9]. Adhesion to the intestinal mucosa would allow colonization, although transient, in human intestinal tract and has been related to the ability to modulate the immune system especially during its development [10].

The established probiotics that meet these criteria are generally lactic acid bacteria (LAB), most commonly *Lactobacillus* and *Bifidobacterium* species, but *Lactococcus*, *Streptococcus*, and *Enterococcus* species, as well as some nonpathogenic strains of *Escherichia*

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coli, and certain yeast strains also qualify [11]. LAB in foods have a long history of safe use [12], and dadih is safe for human consumption for hundreds of years [13].

Dadih is fermented buffalo milk in bamboo tubes by natural lactic acid bacteria and thought to be beneficial for human health due to the presence of natural lactic acid bacteria involved in the fermentation [13]. Our interest in conducting human study on *Enterococcus faecium* IS-27526 was based on *in vitro* tolerance to acid and bile [14], their antimicrobial activity against pathogenic bacteria and antimutagenic properties [15] *in vitro* adhesion and competitiveness against pathogen [7,16] and safety of this strain in animal experiments (unpublished data). The present study was designed to i) investigate the immunomodulatory property of *E. faecium* IS-27526 especially on humoral immune response, ii) to evaluate its effect on bodyweight of pre-school children, and iii) to assess the safety of novel probiotic *E. faecium* IS-27526.

2. Materials and methods

2.1. Strain, culture condition and probiotic preparation

E. faecium IS-27526 was isolated from dadih fermented milk [15] and was identified by 16S rRNA gene sequencing as *E. faecium* (GenBank accession no. EF068251). The LAB isolate was cultured in de Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, UK) for 48 h at 37 °C, harvested by centrifugation, lyophilized and stored in the Functional Foods Forum Culture Collection, University of Turku, Finland. For assays with freeze dried probiotic, lyophilization was prepared at the Center of Biotechnology, Agency for the Assessment and Application of Technology, Serpong, Banten Province, Indonesia. The bacteria was cultivated in MRS broth in a 10 L fermentor for 16 h at 37 °C under aerobic conditions, harvested by centrifugation (3200 × g, 4 °C, 20 min), and washed twice with phosphate-buffered saline (PBS; pH 7.0), frozen at −80 °C, and lyophilized. Purity and viability of the lyophilized cells were tested on MRS agar plates before and during the study period.

2.2. Subjects and experimental methods

The present pilot study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of Faculty of Medicine, University of Indonesia. Written informed consent was obtained from all parents or guardians of the study children.

2.2.1. Subjects

After informed consent, eighty one apparently healthy pre-school children (39 boys and 40 girls, between 15 and 54 months age, mean age 33 months) who lived in Teluk Naga sub District, Tangerang, Banten Province, Indonesia were recruited. Thorough physical examination was conducted on each participant, including a complete medical history by a medical doctor, and anthropometric measurements (bodyweight and height) by 2 trained anthropometrists. Their parents or guardians were interviewed on the morbidity and dietary intake of study children, as well as the household general socio-demographic data, an interviewer administered using the structured questionnaire was collected to find out information on the health condition, and nutritional status of each subject according to WHO [17]. Subject volunteers suffering from inflammatory illnesses or who had been treated with antibiotics in the last two weeks or during study period were excluded.

2.2.2. Design

This pilot study was conducted in a randomized, placebo-controlled, double-blinded pre-post trial. The trial required oral consumption of probiotic *E. faecium* IS-27526 over a total period of 90 days. The 79 subject volunteers met the inclusive criteria were randomly assigned in consecutive way. Among the placebo group ($n = 40$), there were 23 girls and 17 boys, received 1 mg maltodextrin in 125 ml commercial UHT low fat milk, and the probiotic group ($n = 39$), there were 17 girls and 22 boys administrated with 1 mg lyophilized *E. faecium* IS-27526 (2.31×10^8 cfu/day) in 125 ml commercial UHT low fat milk everyday for a period of 90 days. The UHT milk was prepared by Ultra Jaya Milk Co. Bandung, West Java, Indonesia).

The subjects were instructed to abstain from fermented dairy products during intervention, but otherwise maintained their usual diet and lifestyle. Antibiotic consumption two weeks prior to intervention and during experimental days were excluded, and no vaccination procedure was done during the study period. Compliance of intake were monitored and assured by care givers, village midwives from Teluk Naga public health center throughout the study period. Physical examination, morbidity and anthropometric measurements were recorded four times, pre-intervention and at months 1, 2 and 3 during the intervention period. Saliva and blood samples were collected at baseline (control sample) and after 90 days of supplementation (test sample), and kept frozen at −20 °C until being analyzed.

The main parameters studied were total serum IgA and total salivary IgA levels, while the other parameter was the increment of children's bodyweight in probiotic and placebo group by comparing the pre-treatment and post-treatment values. Adverse events and adverse effects such as morbidity, mortality and lowering bodyweight during 90 days supplementation observed to validate the safety of probiotic.

2.3. Probiotic administration

Subjects were administered probiotic or placebo as follows: 1 mg lyophilized *E. faecium* IS-27526 or maltodextrin was added into 125 ml UHT low fat milk in tetra pack packaging, and held for 1 h at room temperature to allow the lyophilized probiotic being viable prior to administrated to the subjects. Daily supplementation in both groups was conducted at Teluk Naga public health center and was given by trained village midwives and care givers with hygiene precaution.

2.4. Blood and saliva collection

Saliva specimen were collected from the mouth on a single occasion directly into sterilized tubes. All saliva samples were centrifuged for 15 min at 10,000 × g, 4 °C to remove cells and debris. Blood were withdrawn by professional nurses, before and after the supplementation. Blood were collected by using disposable wing needle (5 ml). Blood samples were allowed to clot at room temperature for 3 h. Serum was collected by centrifugation at 2500 revolutions per minute (rpm) for 20 min at 4 °C. Serum and supernatant of saliva were kept at −20 °C until used.

2.5. Anthropometric assessment

Weight and height of the children were recorded. A 2-m–long and 1-mm–wide metal tape (Microtoise-Stanley-Mabo Ltd, Poissy, France) was used for height measurement to the nearest 0.1 cm. Weight was measured using a digital scale (UNISCALE, Seca). Children were weighed using indoor light clothing and without shoes and their weight was recorded to the nearest 0.1 kg.

Measurements were taken twice for each child and the average value was used for data entry.

Three anthropometric indices are generally used as proxy measurements for children's nutritional status; height-for-age *Z*-score (HAZ), weight-for-age *Z*-score (WAZ) and weight-for-height *Z*-score (WHZ) [17]. A cutoff of -2SD was used to distinguish normal children from those stunted (HAZ <-2) or underweight (WAZ <-2) or wasted (WHZ <-2). In this study, nutrition status was assessed using the weight-for-age Z score (WAZ).

2.6. Antibody immunoglobulin A (IgA) quantification with enzyme-linked immunosorbent assay (ELISA)

The serum antibody and salivary IgA titers (IgA levels) were measured by sandwich ELISA, modification of Becton Dickinson's ELISA method with horseradish peroxidase. There are four basic steps involved: 1) coating, 2) incubation the test sample and standard, 3) adding a horseradish peroxides-labeled antibody and 4) adding the substrate and indicator dye to detect the positive reaction.

Mouse monoclonal antibody anti-human IgA (Sigma, USA), diluted 1:1000 in carbonate buffer were added at 100 μl/well to coat the 96-wells-Maxisorp microplates flat bottom (Nunc, USA), incubated at room temperature, overnight. After incubation, the plates were washed three times with washing solution PBS-Tween 20 (0.05%). Blocking was performed by use of 100 μl phosphate buffer containing 0.5% bovine serum albumin (BSA) at room temperature for 2 h 15 min and washed three times. Sera was diluted 10 times while saliva was diluted 15 times in PBS.

One hundred microliters of sera or 50 μL of saliva samples (in duplicate) and standard human IgA (Sigma, USA) samples (in duplicate) each was pipetted into microtitre wells. The plates were incubated for 2 h at room temperature. The wells were washed three times with washing solution. Then 100 μl/well of goat anti-human IgA conjugated with horseradish peroxidase (HRP) (Sigma, USA), diluted 1:20,000 in PBS were pipetted into each well, and the plates were incubated at room temperature in the dark for 2 h. The wells were washed three times with washing solution and tapped dry. A fresh substrate solution tetramethylbenzidine (TMB), 100 μl/well was added, and the plates were incubated for 30 min at room temperature in the dark. The enzyme reaction was stopped with 100 μl/wellof1.23 M H_2SO_4 and the optical density (OD) was read by Multiscan ELISA reader at 450 nm (Labsystem, Finland). Samples were analyzed in triplicate. IgA levels were detected by using a standard curve. Salivary IgA levels were quantitated by using appropriate dilution of a standard IgA sample with a known concentration of IgA, provided by the manufacturer (Sigma, USA) and expressed as μg/ml.

2.7. Statistical analysis

Comparison of the means of IgA level within group was tested by paired *t*-test, and to compare the mean changes of IgA level after 90 days supplementation between group, the t test was used. The data were checked for normality by the Kolmogorov–Smirnov test. The mean weight gain was analysed by Mann-whitney, and the mean bodyweight by Wilcoxon tests. The P level of 0.05 was considered as significant [18].

3. Results and discussion

Among the placebo group, twenty subjects were underweight, ten subjects severe underweight and nine subjects were in normal bodyweight, and in probiotic group, twenty two subjects were underweight, seven subjects severe underweight and ten subjects were in normal bodyweight.

Total serum IgA level was not significantly different after 90 days supplementation, between probiotic and placebo group, however, tended to increase in both group. In probiotic group, the total IgA concentrations elevated by (54 ± 185 μg/ml), from 220 ± 132 μg/ml to 274 ± 229 μg/ml, higher than in placebo group (35 ± 250 μg/ml), from 231 ± 124 μg/ml to 267 ± 255 μg/ml. The increase of total serum IgA level in placebo group might be due to the supplementation of 125 ml UHT low fat milk for 90 days as probiotic vehicle.

Total salivary sIgA level in placebo group was increased by 400 ± 136 μg/ml, while in probiotic group was increased by 919 ± 162 μg/ml. The results indicate there is a remarkable higher augmentation of total salivary sIgA level of children in probiotic group compared to placebo group.

Based on their bodyweight, Fig. 1 demonstrates significant elevation of total salivary sIgA of underweight children in probiotic group, higher than the placebo group after 90 days *E. faecium* IS-27526 (10^8 cfu/day) supplementation. It is interesting that *E. faecium* IS-27526 enhances total salivary sIgA level in underweight children.

The augmentation of humoral immune response by *E. faecium* IS-27526 is similar to the adjuvant action previously demonstrated by previous studies. *L. casei* at 10^7 cfu/day improved mucosal immune system and increased sIgA secreting cell significantly in malnourished mouse [19], in line with the results of current study.

The increase of total salivary sIgA level in placebo group might be due to milk supplemented as probiotic carrier given to both groups, placebo and probiotic group. The effect of milk supplementation was significant especially because the children were underweight. However, supplementation of *E. faecium* IS-27526 with milk significantly elevated the changes of salivary sIgA level in underweight children than milk only as shown in Fig. 1.

The first contact between ingested bacteria and the immune system of the host is in the gut-associated lymphoid tissue (GALT). The human intestine is the largest mass of lymphoid tissue in the body. Different components of the mucosal immune system act to focus a specific response against exogenous antigens. sIgA together with the innate mucosal defenses provide the first line of defense against microbial antigens at the intestinal mucosal surface [20]. sIgA inhibits the colonization of pathogenic bacteria in the gut, as well as the mucosal penetration of pathogenic antigens [21]. The ability of probiotics to modulate IgA concentrations in the gut has been the subject of several studies. The results obtained in the present study showed that the increment of sIgA levels were

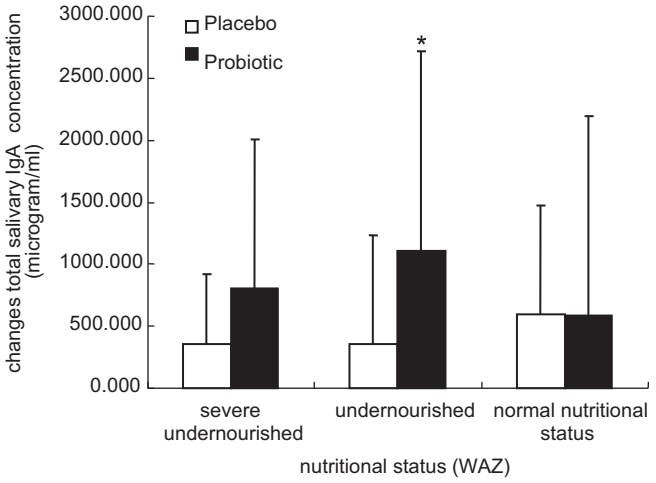

Fig. 1. The mean elevated values of total salivary IgA levels (μg/ml) based on their nutritional status, weight-for-age Z score (WAZ).

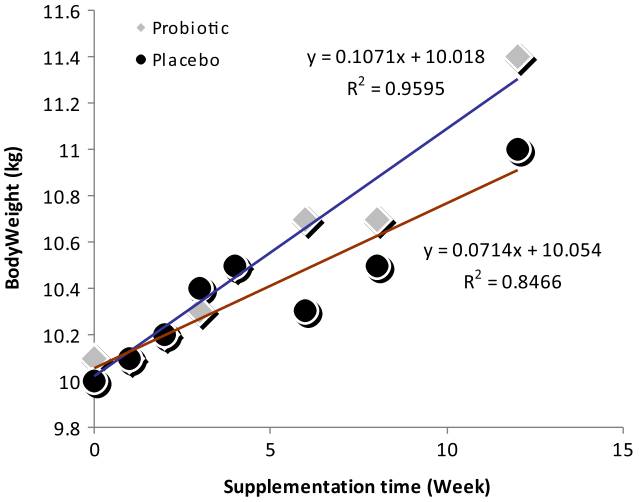

Fig. 2. Linear regression of bodyweight in both groups. Y1 represents placebo group, and y2 represents probiotic group.

significantly higher in underweight children of probiotic group than placebo group.

E. faecium IS-27526 has been shown to adhere to human intestinal mucus and has ability to inhibit adhesion, displace previously adhered pathogens, and compete for adhesion sites in intestinal human mucus [16]. *E. faecium* IS-27526 appeared to have good autoaggregation abilities [7]. Adhesion to intestinal mucosa has been reported for most of the current probiotic strains with demonstrated clinical efficacy. Adhesion may allow colonization by the specific strain in human gastrointestinal tract. Colonization promotes the production of sIgA. The most likely explanation for the enhancement of the sIgA response is the adhesion as well as colonization properties of *E. faecium* IS-27526. Bacterial aggregation could be the factor in promoting the gut colonization of beneficial microorganisms [22,23].

Salivary IgA (sIgA) are produced mainly by the Mucosa Associated Lymphoid Tissue (MALT) and better reflect intestinal immune response than monomeric IgA (serum IgA). The best sample for assessment of sIgA is obtained from gut lavage fluid or saliva. Although saliva may not be the optimal source to reflect the mucosal immune response, saliva was easy to obtain from human and reflective of MALT activity [24].

In order to influence the immune system, a probiotic must activate the lymphoid cell in the MALT. It has also been reported that cell wall peptidoglycan is known for a long time to have an adjuvant activity. These structures have been found to be involved in the adhesion of LAB to the intestinal mucosal surface [25].

There are concerns about the use of enterococci for human probiotic purposes. However, in our *in vivo* study, there was no adverse effect recorded in Wistar rats administrated with *E. faecium* IS-27256 (data not shown) and this study also reveals that there was no adverse effect in supplementing *E. faecium* IS-27256 to immunocompromised subjects, underweight children, as shown by no lowering bodyweight, and neither gastroenteritis morbidity nor mortalilty was observed. Hence, safety of novel probiotic *E. faecium* IS-27256 has been validated.

The dominant microorganism in human intestine may play a role in inhibiting the growth of pathogens [26,27]. Some specific components of microbiota also have been associated with beneficial effects of the host, such as promotion of gut maturation and integrity, antagonisms against pathogens and immune modulation [28]. Their absence or low viability may cause varying degrees of digestive problems.

Probiotics have been designed to modify the balance of the existing flora by directly providing high quantities of microbes belonging to species that are normal inhabitants of the intestinal microflora [29]. Probiotics and their metabolite activity have been found to have beneficial health effect such as, stabilization the intestinal microbiota, antagonisms against pathogens, and increase immune response that may lead to increase the general health.

The ability of this probiotic strain in inhibiting pathogens may maintain the integrity of epithelial cell of the intestine so that the nutrient absorption might be optimum in normal bodyweight children, as a consequence, the weight gain in normal bodyweight children supplemented with *E. faecium* IS-27526 for 90 days significantly higher than the placebo group. Bodyweight is a good parameter to know the general health condition of the host in short time periods of intervention.

The mean bodyweight gain in probiotic group was 1.28 ± 0.94 kg, from 10.13 ± 1.22 kgto 11.41 ± 1.31 kg, while in placebo group was 0.99 ± 0.99 kg from 10.01 ± 1.58 kg to 11.0 ± 1.87 kg.

Fig. 2 shows the linear regression for placebo group, $y = 0.0714x + 10.054$, $R2 = 0.8466$ while in probiotic group, $y = 0.1071x + 10.018$, $R2 = 0.9595$. *Y* axis represents bodyweight (kg) andx axis represents supplementation time (week). The slope or regression coeficient at probiotic group is (0.107), bigger than placebo group (0.0714), means that in every one week supplementation the bodyweight of children in probiotic group may increase by 0.107 kg, while the bodyweight of children in placebo

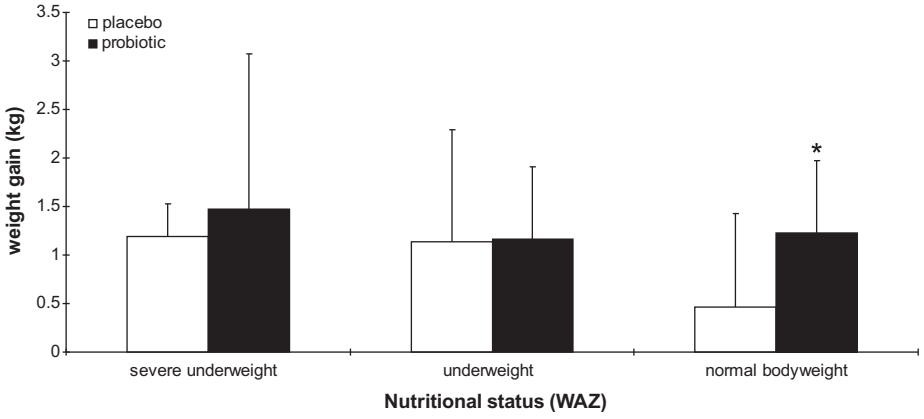

Fig. 3. Weight gain based on nutritional status, weight-for-age Z score (WAZ).

group may increase by 0.0714 kg. Supplementation of *E. faecium* IS-27526 at 10^8 cfu/day and 125 ml low fat milk for 90 days may increase the bodyweight of children by 1.5 times higher than supplementation of 125 ml low fat milk only.

Based on the nutritional status of subjects, weight gain was found to be significant in children with normal bodyweight supplemented with probiotic (Fig. 3), and might be due to the adhesion and colonization of *E. faecium* IS-27256, which may maintain the integrity of intestine, so that facilitating optimum nutrient absorption. Hence, the health promoting effects might be related to the biological activity of probiotic bacteria. A relation between *in vitro* adhesion and *in vivo* colonization has been reported [12]. In addition, adhesion to and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients or immune modulation [13]. The same strain was also previously studied for its *in vitro* and *in vivo* probiotic attributes such as resistance to acidity, bile tolerance, surface hydrophobicity, aggregation adhesion properties, and competitiveness against pathogens [14,7,16], and this human study confirmed the probiotic function of *E. faecium* IS-27256 in human.

4. Conclusion

Taken together, *E. faecium* IS-27526 together with milk was significant in augmenting the salivary sIgA level in underweight children, and gaining weight of children with normal bodyweight. Moreover, safety has been validated in vulnerable population such as underweight young children. Hence, *E. faecium* IS-27526 hold great promise as potential novel probiotic strain in stimulating the total salivary sIgA level better than milk only in underweight preschool children. The increase of secretory IgA level is important to protect the mucosal surface from infectious diseases.

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References

[1] Suau A, Bonnet R, Sutren M, Godon J-J, Gibson G, Collins MD, et al. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol 1999;65:4799–807.
[2] Kelly D, Conway S, Aminov R. Commensal gut bacteria: mechanisms of immune modulation. Trends Immunol 2005;26:326–33.
[3] Spiller R, Garsed K. Infection, inflammation, and the irritable bowel syndrome. Dig Liver Dis 2009;41(12):844–9.
[4] Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm 2009;15:1546–58.
[5] Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr 1999;69: 1052S–7S.
[6] Fuller R, Gibson GR. Modification of the intestinal microflora using probiotics and prebiotics. Scand. J Gastroenterol Suppl 1997;222:28–31.
[7] Food and Health Agricultural Organization of the United Nations and World Health Organization. Guidelines for the evaluation of probiotics in food. Working group rep. Washington DC: Food and Health Agricultural Organization of the United Nations and World Health Organization; 2002.
[8] Collado MC, Gueimonde M, Hernandez M, Sanz YM, Salminen S. Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and its role in enteropathogen exclusion. J Food Prot 2005;68(12):2672–8.
[9] Collado MC, Surono IS, Meriluoto J, Salminen S. Indigenous dadih lactic acid bacteria: cell-surface properties and Interactions with pathogens. J Food Sci 2007;72(3):M89–93.
[10] Schiffrin EJ, Brassard D, Servin AL, Rochat F, Donnet-Hughes A. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am J Clin Nutr 1997;66:515–20.
[11] Borchers MT, Wesselkamper SC, Deshmukh H, Beckman E, Medvedovic M, Sartor M, et al. The role of T cells in the regulation of acrolein-induced pulmonary inflammation and epithelial-cell pathology. HEI health review committee. Res Rep Health Eff Inst 2009;146:5–29.
[12] Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, et al. Demonstration of safety of probiotics – a review. Int J Food Microbiol 1998; 44:93–106.
[13] Akuzawa R, Surono IS. Fermented milks of Asia. In: Roginski H, Fuquay JW, Fox PF, editors. Encyclopedia of dairy sciences. London, UK: Academic Press Ltd.; 2002. p. 1045–8.
[14] Surono IS. *In vitro* probiotic properties of indigenous dadih lactic acid bacteria. Asian Aus J Anim Sci 2003;16:726–31.
[15] Surono IS, Hosono A. Antimutagenicity of milk cultured with lactic acid bacteria from dadih against mutagenic terasi. Milchwissenschaft 1996;51: 493–7.
[16] Collado MC, Surono IS, Meriluoto J, Salminen S. Potential probiotic characteristics of *Lactobacillus* and *Enterococcus* strains isolated from traditional dadih fermented milk against pathogen intestinal colonization. J Food Prot 2007;70(3):700–5.
[17] WHO. Anthro beta version Feb 17th, 2006: software for assessing growth and development of the world's children [cited 2006 Jul 10]. Available from: Geneva: WHO http://www.who.int/childgrowth/software/en/; 2006.
[18] Minium EW, Clarke RC, Coladarci T. Elements of statistical reasoning. 2nd ed. New York: John Wiley & Sons; 1999.
[19] Perdigon G, Vintini E, Alvarez S, Medina M, Medici M. Study of the possible mechanisms involved in the mucosal immune system activation by lactic acid bacteria. J Dairy Sci 1999;82(6):1108–14.
[20] Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents – physiological effects and clinical benefits. Aliment Pharmacol Ther 2005;22:495–512.
[21] Adolfsson O, Meydani SN, Russell RM. Yogurt and gut function. Am J Clin Nutr 2004;80(2):245–56.
[22] Cesena C, Morelli L, Alander M, Siljander T, Tuomola E, Salminen S, et al. *Lactobacillus crispatus* and its nonaggregating mutant in human colonization trials. J Dairy Sci 2001;84:1001–10.
[23] Jankovic I, Ventura M, Meylan V, Rouvet M, Elli M, Zink R. Contribution of aggregation-promoting factor to maintenance of cell shape in *Lactobacillus gasseri* 4B2. J Bacteriol 2003;185:3288–96.
[24] Erickson KL, Hubbard NE. Probiotic immunomodulation in health and disease. J Nutr 2000;130(suppl):403S–9S.
[25] Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S. Probiotics: mechanism and established effects. Int Dairy J 1999;9:43–52.
[26] Roitt I, Delves PJ. Roitt's essential immnuology. 10th ed. London: Blockwell Scientific Publication; 2001.
[27] Jelen P, Lutz S. Functional milk and diary products. In: Mazza G, editor. Functional foods: biochemical & processing aspects. Lancaster, Basel: Technomic Publishing Co. Inc.; 1998.
[28] Brassart D, Schiffrin EJ. Pre- and probiotics. In: Scmidl MK, Labuza TP, editors. Essentials of functional foods. Gaithersburg, Maryland: An Aspen Publication; 2000.
[29] Varnam AH, Sutherland JP. Milk and milk products: technology, chemistry and microbiology. London: Chapman & Hall; 1994.