# Identification and Characterization of Probiotic Lactic Acid Bacteria Isolated from Indigenous Goat Milk

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**Abstract.** Probiotic lactic acid bacteria play role as functional food and it is very important to know their identification and characterization. The diversity of lactic acid bacteria isolated from Ettawa crossbred and Saanen crossbreed goat milk was studied in terms of morphology, physiology and their characteristics. A total of 33 lactic acid bacterial strains were isolated and 18 isolates passed the initial selection. The second step was in vitro test, namely their resistances to low pH (2, 2.5, and 3.5) and bile salt (0.3%) were evaluated to know their potential as probiotic. The results showed that all strains grew well at acid condition (pH 2, 2.5, and 3.2) and seven strains grew well at bile salt (0.3%). Identification with API test for seven isolates showed that two isolates were *Lactobacillus rhamnosus*, 1 isolate was *Lactobacillus plantarum* and four isolates were *Lactobacillus plantarum* 1.

**Key Words**: goat milk, lactic acid bacteria, probiotic, in vitro

## Introduction

Probiotic lactic acid bacteria are often found naturally in foodstuffs such as meat, milk, and vegetables. Probiotic represents all species of microorganisms that are beneficial if they are consumed in living condition and proper number. While in the processed food products, the number of probiotic bacteria should be $10^6$-$10^7$ CFU/g or $10^8$-$10^9$ CFU in 100 g or 100 ml daily food consumption in order to get the medicinal benefit (Jayamanne and Adams, 2006). This is because the microbes must be able to survive against great stomach and intestinal conditions; therefore, they are effective to keep the equilibrium of intestine microbial population. Milk and its processed products represent one of the growth media for the LAB. There are great numbers of probiotic lactic acid bacterium explorations to obtain starter cultures that are useful to yield functional fermentation products in order to maintain health.

Lactic acid bacterium identifications can be conducted via its morphology and biochemistry tests. Its morphological characteristic's identification is then conducted to know the genus of the bacterium, which includes Gram coloring and its physiological characterization which includes catalyses test (Sujana, 2008), its survivals at various temperatures, pHs, salinities, $CO_2$, dextrans, and $NH_3$. While to determine the kind of species, its ability to ferment various kinds of sugars (carbohydrates) is tested using API test. The pre-conditions of a probiotic lactic acid bacteria, besides its mandatory to be in the category of GRAS (Generally Recognized as Safe), it should also be recognized as having excellent survival in gastric acid and bile salt conditions (Rasyid et al., 2007; Khalil et al., 2007 and Abdelbasset and Djamila, 2008).

## Materials and Methods

The principal material in this study was goat milk from Ettawa crossbreed (PE) and Saanen crossbreed (PESA). The MRS broth and de-Man Rogosa Sharp Agar media were used. In-vitro characteristic test was conducted using 0.1 N

HCl, oxgall bile salt, and Phosphat Buffer Saline (PBS). The implements used in this study were pH meter, autoclave, centrifuge, magnetic stirer, vortex, cooling box, digital scale, API 50 CHL and various kinds of glass wares.

This study was conducted in 2 phases, namely (1) isolation and identification of LAB and (2) characterization of probiotic LAB using i*n-vitro* test.

**Isolation and identification of LAB**

This study was initiated using goat milk sample collection, with a working procedure as follows (Sujaya et al., 2008): the sample was taken aseptically as much as 1 ml, enriched with 100 ml of liquid MRS (MRSB) and was icubated anaerobically at 37$^{o}$C for 24 hours. Stratified dilution was conducted thereafter, followed by dispersion of 0.1 ml of the solution at MRS agar medium that contained purple bromocresol in petri dish. The next step, the petri dish was incubated at similar condition as above. The LAB colony appeared as a colony surrounded by a yellow-color zone, then it was isolated and streaked on the MRS agar medium. The streak was conducted continuously, until a uniform colony was obtained.

At the first step after isolation, morphological characteristic test was done which included Gram coloring (Prescott, 2002) and physiological characteristic of LAB tests (Harrigan, 1998) that included catalyses, growth at various temperatures (10, 37, and 45$^{o}$C), survival at various pHs (4.0, 7.0, and 9.6), survival at various salinities (4 and 6.5%), and ability to produce $CO_2$ gas, dextran, and $NH_3$. The results of physiology test were compared to those recommended by Axellson Table (2004). The identification of LAB isolates was conducted using API 50 CHL (Conter et al., 2005) consisted of 50 micro tubes that contained carbohydrates and their derivatives (heterocide, poly-alcohol, uronic acid). The first tube did not contain active ingredient and was used as the negative control. The observed parameters were the change of color after 48-hour incubation period. The change of color occurs because of the aerobic production of acids that can be detected by pH indicator of the selected medium. The results of the observation were then processed using Apiweb software ™.

**Characterization of probiotic LAB by in-vitro**

The in-vitro tested probiotic LAB characterizations were as follows: test on their survival against low pH and bile salt (Lin et al*.,* 2006). The survival test against low pH was conducted according to the method of 1 ml, 24-hour old LAB culture was poured into 9 ml PBS at pH 2, 2.5, and 3.2. The pH of the pre-used medium was manipulated by using 0.1 N HCl, followed by 3-hour incubation (Gropper and Groff, 2001). Before and after incubation, the numbers of cells was count by using MRSA pouring method, followed by a 37$^{o}$C, 48-hour incubation .

The test toward bacterium survival against bile salt was conducetd as follows: 1 ml, 24-hour old LAB culture was pipetted into 9 ml of MRSB medium that contained 0.3% bile salt (Oxgall). The control medium was MRSB without bile salt, thereafter, both media were incubated at 37$^{o}$C for 48 hours, followed by cell counting using MRSA pouring method.

**Data analysis**

The data of LAB tests on their survivals against pH and bile salt were statistically processed by applying one way analysis of variances, followed by Duncan test.

## Results and Discussion

**Morphology, physiology and biochemical characteristics of LAB isolates**

The isolation of LAB from the milk of Ettawa crossbreed (PE) resulted in 16 isolates had been identified, whereas the isolation of LAB from the milk of Saanen crossbreed (PESA) produced 17 isolates (Table 1).

The results of Gram coloring showed that 33 LAB isolates from the milks of PE and PESA were positive Gram bacteria of rod shape (26 isolates), of short rod shape (1 isolate), of round, oval shape (3 isolats), and of round shape (3 isolates) with the characteristic of negative catalase. According to Salminen et al. (2004), LAB are positive Gram bacteria in the shapes of rod or round and have the characteristic of negative catalase. All of the isolates in this study were able to grow at 37$^{o}$C (100%) and 24 isolates at 45$^{o}$C (73 %), however, those that were able to survive at 10$^{o}$C were only 18 isolates (54,5%). The test of LAB tolerances toward salt concentrations of 4 and 6.5 % showed that all isolates of LAB were capable of surviving for 7 days. All of the LAB isolates were able to survive at alkali pH (pH 9.6), however, only 17 isolates (52%) that were able to survive at acidic pH (pH 4.4).

Based on $CO_2$ production, 20 isolates (61 %) had a hetero-fermentative characteristic, whereas 13 isolates (39%) had a homo-fermentative characteristic. The test of $NH_3$ production that was origined from arginine showed that no isolates showed any positive results. This case was beneficial because $NH_3$ production yields fermentative products that have undesirable ammonia odor. The round-shape LAB isolates did not yield any dextrin, therefore, it could be concluded that the isolates were not included in the category of *Leuconostoc*. One of the characteristics of *Leuconostoc* is the production of dextran that is viable as mucous. Dextran is defined as water-soluble poly-glucosaccharide that is formed from α1-6 glucosidal bond with a proportion range of 0-20 %. In this study, as many as 18 LAB isolates were identified as *Lactobacillus.* The isolates were further tested to evaluate their potential as probiotic via survival tests against low pH as well as bile salt.

**Identification of LAB isolates using API 50 CHL**

There were only 7 LAB isolates that were identified using API 50 CHL, namely LAB isolates that had good survival against bile salt; isolate number 2, 3, 4, 10, 14, 26 and 28. The seven isolates had positive fermentative characteristics to ribose, glucose, fructose, manose, N-acetylglucosamin, amigdalin, arbutin, esculin, salisin, sellobiose, maltose, lactose, threhalose and B-getibiose. The ability of LAB isolates to ferment oligosaccharides represents one of the desirable probiotic characteristics because the mono saccharine that exist in the gastro-intestinal tract will affect the life of micro-organisms in the intestine (Kaplan and Hutkins, 2000).

On the basis of reference strains (bioMérieux, 2006), isolates number 2 and 3 were identified as *Lactobacillus rhamnosus* with the similarity levels of 99.9 and 99.3%, respectively. Isolate number 4 was identified as *Lactobacillus plantarum* with the degree of acidity of 89.2 %, while isolates number 10, 14, 26 and 28 were identified as *Lactobacillus plantarum* 1 with the degree of acidity of 99,9 % for each isolate.

**The survival of LAB isolates against low pH and bile salt**

The results of this study revealed that all the tested LAB isolates were able to survive at pH 2.0, 2.5 and 3.2. The decrease of LAB colony forming unit (CFU) at the low pH condition was lower than 1.0 log unit (Table 2). The results indicated the high ability of LAB isolates from goat milk to survive through acidic, great stomach (gastric tract). The results of this study was in line with those of Minellia et al. (2004), who found out that four strains of probiotics were able to survive at pH 2 for as long as 2 hours and were able to grow at pH 3.0 and 4.0. The characteristics of LAB, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* against low pH were also studied by Kalaui et al. (2007). He found out that all the tested isolates were able to survive at pH 2.0 that lasted for 3 hours. At the application level,

Table1. Physiological characteristics of LAB isolates from goat's milk

| No. | The origin of isolates | Isolate code | Catalase | Temperature (°C) | | | Salt concentration (%) | | | pH | | | Production | | | Initial identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 | 37 | 45 | 4 | 0 | 6.5 | 4.4 | 7.0 | 9.6 | CO2 | dextran | NH3 | |
| 1 | PE | 1 | - | + | + | - | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 2 | PE | 2 | - | + | + | + | + | + | + | - | + | + | - | - | - | *L.* homofermentatif |
| 3 | PE | 3 | - | + | + | + | + | + | + | + | + | + | - | - | - | *L.* homofermentatif |
| 4 | PE | 5 | - | + | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 5 | PE | 6 | - | - | + | - | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 6 | PE | 9 | - | + | + | + | + | + | + | - | + | - | + | - | - | *L.*heterofermentatif |
| 7 | PE | 11 | - | + | + | + | + | + | + | + | + | + | - | - | - | *L.* homofermentatif |
| 8 | PE | 13 | - | - | + | + | + | + | + | - | + | + | - | - | - | *S.*homofermentatif |
| 9 | PE | 19 | - | - | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 10 | PE | 22 | - | - | + | + | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 11 | PE | 23 | - | - | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 12 | PE | 26 | - | + | + | + | + | + | + | - | + | + | - | - | - | *S.*homofermentatif |
| 13 | PE | 28 | - | + | + | + | + | + | + | + | + | + | - | - | - | *L.* homofermentatif |
| 14 | PE | 29 | - | + | + | + | + | + | + | + | + | + | + | - | - | *S.*heterofermentatif |
| 15 | PE | 31 | - | + | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 16 | PE | 32 | - | + | + | + | + | + | + | - | + | + | - | - | - | *L.* homofermentatif |
| 17 | PESA | 4 | - | + | + | + | + | + | + | + | + | + | - | - | - | *L.* homofermentatif |
| 18 | PESA | 8 | - | - | + | - | + | + | + | - | + | + | - | - | - | *L.* homofermentatif |
| 19 | PESA | 10 | - | + | + | + | + | + | + | - | + | + | - | - | - | *L.* homofermentatif |
| 20 | PESA | 12 | - | - | + | - | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 21 | PESA | 14 | - | + | + | + | + | + | + | - | + | + | - | - | - | *L.* homofermentatif |
| 22 | PESA | 15 | - | + | + | + | + | + | + | + | + | + | - | - | - | *L.* homofermentatif |
| 23 | PESA | 16 | - | - | + | - | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 24 | PESA | 17 | - | + | + | + | + | + | + | + | + | + | + | - | - | *S.*heterofermentatif |
| 25 | PESA | 18 | - | - | + | - | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 26 | PESA | 20 | - | - | + | + | + | + | + | - | + | + | + | - | - | *L.*heterofermentatif |
| 27 | PESA | 21 | - | - | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 28 | PESA | 24 | - | + | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 29 | PESA | 25 | - | - | + | - | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 30 | PESA | K11 | - | + | + | + | + | + | + | - | + | + | - | - | - | *S.* homofermentatif |
| 31 | PESA | 33 | - | - | + | - | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |
| 32 | PESA | 34 | - | + | + | - | + | + | + | - | + | + | + | - | - | *S.*heterofermentatif |
| 33 | PESA | 35 | - | - | + | + | + | + | + | + | + | + | + | - | - | *L.*heterofermentatif |

if LAB enter into human body, the first constrain is when they are exposed to gastric acid with very low pH level, around 2 for the empty stomach and 3 for the full one (Martini et al., 1997). The LAB survival increases with the existence of food in the stomach, which affects pH and there is a possibility that the full stomach condition will protect LAB from degradations by gastric acid and pepsin enzyme. Some commercial strains of LAB have several differences in their survival ability against low pH (Lin et al., 2006). The LAB tolerances against acids are due to their abilities to keep the constant, higher alkalinity of cytoplasm relative to that of extracellular condition (Hutkins and Nannen, 1993). The LAB tolerance against acids is very important to withstand initial stress in the stomach, on the

Tabel 2. The decrease in the number (log cfu/ml) of LAB isolate colonies at low pH and 0.3% bile acid condition

| No. | LAB Code | The decrease of colony (log cfu/ml) | | | |
|---|---|---|---|---|---|
| | | pH 2.0 | pH 2.5 | pH 3.2 | 0.3 % of bile salt |
| 1. | 2 | 0.94±0.03 $^{f}$ | 0.14±0.11 $^{cd}$ | 0.16±0.13 $^{fg}$ | 1.70±0.16 $^{a}$ |
| 2. | 3 | 0.27±0.03 $^{cd}$ | -0.15±0.07 $^{ab}$ | 0.19±0.02 $^{fg}$ | 2.48±1.22 $^{c}$ |
| 3. | 4 | 0.18 ±0.05 $^{bc}$ | 0.46±0.04 $^{e}$ | 0.15±0.13 $^{efgh}$ | 2.11±0.83 $^{a}$ |
| 4. | 8 | 0.25±0.004 $^{cd}$ | 0.01±0.04 $^{bcd}$ | -0.23±0.06 $^{abc}$ | 4.31±0.38 $^{c}$ |
| 5. | 10 | 0.03±0.11 $^{ab}$ | 0.07±0.14 $^{bcd}$ | 0.13±0.07 $^{efgh}$ | 1.70±0.42 $^{a}$ |
| 6. | 14 | -0.04±0.13 $^{a}$ | 0.29±0.14 $^{de}$ | 0.05±0.06 $^{defg}$ | 1.81±0.40 $^{a}$ |
| 7. | 15 | 0.13±0.03 $^{cd}$ | 0.05±0.09 $^{bcd}$ | -0.26±0.02 $^{ab}$ | 4.17±0.47 $^{c}$ |
| 8. | 19 | 0.51±0.01 $^{de}$ | -0.03±0.01 $^{bcd}$ | -0.308±0.02 $^{a}$ | 4.43±0.62 $^{c}$ |
| 9. | 21 | 0.16±0.09 $^{bc}$ | 0.06±0.11 $^{bcd}$ | -0.08±0.07 $^{bcd}$ | 4.60±0.37 $^{c}$ |
| 10. | 22 | 0.59±0.12 $^{abc}$ | -0.07±0.21 $^{bcd}$ | 0.05±0.11 $^{defg}$ | 4.52±0.20 $^{c}$ |
| 11. | 23 | 0.13±0.17 $^{abc}$ | -0.02±0.06 $^{bcd}$ | -0.04±0.13 $^{de}$ | 4.50±0.53 $^{c}$ |
| 12. | 24 | 0.04±0.02 $^{abc}$ | 0.04±0.02 $^{bcd}$ | 0.04±0.12 $^{defg}$ | 4.63±0.04 $^{c}$ |
| 13. | 26 | 0.96±0.08 $^{f}$ | -0.06±0.19 $^{bcd}$ | -0.103±0.01 $^{bcd}$ | 1.74±0.02 $^{a}$ |
| 14. | 28 | 0.19±0.13 $^{bc}$ | -0.07±0.06 $^{bcd}$ | -0.13±0.08 $^{abcd}$ | 2.75±0.19 $^{ab}$ |
| 15. | 29 | 0.58±0.15 $^{e}$ | 0.44±0.01 $^{e}$ | 0.25±0.04 $^{h}$ | 4.53±0.01 $^{c}$ |
| 16. | 32 | -0.06±0.09 $^{ab}$ | -0.33±0.01 $^{a}$ | -0.16±0.02 $^{abc}$ | 3.61±0.57 $^{bc}$ |
| 17. | 33 | 0.18±0.06 $^{bc}$ | 0.14±0.04 $^{cd}$ | -0.08±0.07 $^{bcd}$ | 3.57±0.10 $^{bc}$ |
| 18. | 35 | 0.26±0.17 $^{cd}$ | 0.04±0.04 $^{bcd}$ | 0.20±0.01 $^{fg}$ | 3.12±0.68 $^{bc}$ |

Values bearing different superscript at the same column differ significantly (P<0.05)
(+): there was a decrease in the number of microbial colony; (-): there was an increase in the number of microbial colony

other side; acid condition in the long run is required by the LAB as a carrier of food such as in yoghurt (Minellia et al., 2004). Beside of acid, physical and chemical characteristics of food *carrier* also function as buffer and affect the survival of LAB in the digestive tract (Patel et al., 2004).

After being exposed to the acidic pH, the LAB isolates were further tested for their survival against bile salt (Table 2). As many as 7 isolates out of 18 (39%) were able to survive 0.3% of bile salt. Isolate of numbers 2, 3, 4, 10, 14, 26 and 28 only experienced the decrease in the number of cfu as much as 1-2 log, therefore, it could be stated that LAB isolates were able to survive at bile salt condition. The survival at bile acid condition is one of the critical points for the microbes. Several strains of *Lactobacillus* are able to hydrolyze bile salt by using specific enzymes, bile salt hydrolysis, that is able to decrease the solubility of bile salt, which in turn, decreases or eliminates the toxic effect of the bile salt to the LAB. This case is one of the factors, why some LAB isolates are capable of surviving at bile salt condition. Whereas 3 LAB isolates experienced a decrease of colony number as much as 3 log, namely isolates of numbers 32, 33 and 35, the others experienced a decrease in the number of colony as much as 4 log. According to Succi et al. (2005), the decrease in survival of LAB at bile salt condition, is due to the ability of bile salt that is secreted to the intestine to destroy the main components of cell membrane, fat and fatty acids, of the LAB and in turn, it affects the permeability of cell membrane and viability of LAB. On the other side, there are interactions between cell membrane with its environment. A further study by Erkkilae and Petaeja (2000) to test *L. curvatus* and *P. acidilactici* revealed that the bacteria were also able to survive against acid and 0.3% bile salt. In addition, Burns et al. (2008) compared the survival against bile salt of two *Lactobacillus* strains, one of which was collected from digestive tract and another one was from milk product. Their results showed that *Lactobacillus* that was isolated from digestive tract was more resistant

relative to *Lactobacillus* that was isolated from the milk product.

## Conclusions

This study has successfully identified 16 LAB isolates from Ettawa crossbreed (PE) goat and 17 isolates of LAB from the milk of Saanen crossbreed (PESA) goat. Physiological test indicates that all LAB isolates have negative catalase characteristic, are able to grow at 37 and 24 isolates at 45$^{o}$C (73%), however, there are only 18 isolates (54, 5%) that are able to survive at 10$^{o}$C. All LAB isolates are able to survive up to 7 days in salt concentrations of 4 and 6.5 %, survive at alkali pH (pH 9.6) and only 15 isolates (45%) that are capable of surviving at acidic pH (pH 4.4). Twenty LAB isolates have the hetero-fermentative characteristic (61 %) and 13 isolates (39 %) are homo-fermentative. All isolates do not produce dextran and $NH_3$ from arginine and 18 isolates are able to survive at pH 2.0, 2.5, and 3.2 with the decrease in the number of microbial colony of less than 1 log unit. As many as seven isolates are able to survive in 0.3% of bile salt, namely number 2, 3, 4, 10, 14, 26, and 28 with the decrease in the number of microbial colony, in the range of 1-2 log units.

The results of genus and species identifications by using API show that the potential isolates as probiotic LAB are *Lactobacillus rhamnosus* (isolates of number 2 and 3), *Lactobacillus plantarum* (isolate number 4) and *Lactobacillus plantarum* 1 (isolates of number 10, 14, 26 and 28).

It is recommended that in-vivo test is needed to evaluate the seven isolates that have potential as probiotics, therefore, the isolates can be applied and used as the starter cultures for functional food.

## References

Abdelbasset M and K Djamila. 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from algerian traditional fermented milk "Raib". African J. Biotech. 7:2908-2914.

Araújo EA, AF de Carvalho, ES Leandro, MM Furtado and CA de Moraes. 2010. Development of a symbiotic cottage cheese added with *Lactobacillus Delbrueckii Ufv H2b20* and inulin. J. Functional Foods. 2:85-89.

Axelsson L. 2004. Lactic Acid Bacteria: Classification and Physiology. In: Salminen, S. and Von Wright A. (Eds.). Lactic Acid Bacteria: Microbiology and Functional Aspects. Marcel Dekker Inc, NY.

Burns PF, D Patrignani, Serrazanetti, GC Vinderola, JA Reinheimer, R Lanciotti and ME Guerzoni. 2008. Probiotic crescenza cheese containing *Lactobacillus casei* and *Lactobacillus acidophilus* manufactured with high-pressure homogenized milk. J. Dairy Sci. 91:500–512.

Conter M, T Muscariello, E Zanardi, S Ghidini, A Vergara, G Campanini and A Ianieri. 2005. Characterization of lactic acid bacteria isolated from an italian dry fermented sausage. Ann. Fac. Medic. Vet. Parma. 25:167-174.

Erkkilae SE and Petaeja. 2000. Screening of commercial meat starter cultures at low ph and in the presence of bile salts for potential probiotic use. Meat Science. 55:297-300.

FAO/WHO (Food Agriculture Organization/World Health Organization). 2002. Guidelines for Evalution of Probiotic in Food. Report of Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotic in Food. London Ontario, Canada.

Gropper SS and Groff. 2001. Advanced Nutrition and Human Metabolism 3 $^{Rd}$ Ed. Wadswoth, USA.

Harrigan WF. 1998. Laboratory Methods in Food Microbiology . Academic Press Inc. New York.

Hutkins RW and NL Nannen. 1993. pH homeostasis in lactic acid bacterial. J. Dairy Sci. 76:2354-2365.

Jacobsen CN, R Nielsen and AE Hayford. 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus Spp*, in-vitro technicques and evaluation of the colonization ability of five selected strains in human. Applied and Enviromental Microbiol. 65:4949–4956.

Jayamanne VS and MR Adams. 2006. Determination of survival, identity, and stress resistance of probiotic bifidobacteria in bio-yoghurts. Letters in Applied Microbiology. 42:189-194.

Kalui CM, M Julius, M Philip, Kutima, C Kiiyukia and LE Wongo. 2009. Functional characteristics of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* from Ikii, a Kenyan traditional fermented maize porridge. African J. Biotech. 18:4363-4373.

Kaplan H and RW Hutkins. 2000. Fermentation of fructooligosaccha-rides by lactic acid bacteria and bifidobacteria. Applied and Enviromental Microbiol. 66:2682-2684.

Khalil R, H Mahrous, K El-Halafawy, K Kamaly, J Frank and ME Soda. 2007. Evaluation of the probiotic potential of lactic acid bacteria isolated from feces of breast-fed infants in Egypt. African J. Biotech. 6:935-945.

Lin WH, CFH Wang, LW Chen and HY Tsen. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria product. Food Microbiol. 23:78–81.

Martini MC, GL Bolweg, MD Levitt and DA Savaiano. 1987. Lactose digestion by yoghurt h-galactosidase influence of pH and microbial cell integrity. American J. Clinic. Nut. 45:432–437.

Minellia EB, A Beninia, M Marzottob, A Sbarbatic, O Ruzzenented, R Ferrarioe, H Hendriksf and F Dellaglio. 2004. Assessment of novel probiotic *Lactobacillus casei* strains for the production of functional dairy foods. International Dairy J. 14:723–736.

Patel HM, SS Pandiella, RH Wang and C Webb. 2004. Influence of malt, wheat and barley extracts on the bile tolerance of selected strains of lactobacilli. Food Microbiol. 21:83-89.

Prescott H. 2002. Laboratory Exercises in Microbiology. 5 th ed. McGraw-Hill Companies.

Rasyid H, K Togo, M Ueda and T Miyamoto. 2007. Probiotic characteristics of lactic acid bacteria isolated from traditional fermented milk 'Dahi' in Bangladesh. Pakistan J. Nut. 6:647–652.

Salminen S, AV Wright and A Ouwehand. 2004. Lactic Acid Bacteria 3rd ed, Marcel Dekker Inc. New York.

Succi M, P Tremonte, A Reale, E Sorrentino, L Grazia, B Pacifino and R Coppola. 2005. Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolates from Parmigiano reggiano cheese. Fems Microbiol. Lett. 244:129-137.

Sujaya N, Y Ramona, NP Widarini, NP Suariani, NM Utama Dwipayanti, KA Nocianitri dan NW Nursini. 2008. Isolasi dan karakterisasi bakteri asam laktat dari susu Kuda Sumbawa. J. Veteriner. 9:52-59.