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# Biochemical Characteristics of Lactic Acid Bacteria with Proteolytic Activity and Capability as Starter Culture Isolated From Spontaneous Fermented Local Goat Milk

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## Abstract

This study was aimed to isolate Lactic Acid Bacteria (LAB) from spontaneus fermented local goat milk, which had proteolytic activity and used for futher fermentation of goat milk. Isolation of LAB was carried out by MRS Agar and $CaCo_3$. Identification species of isolates was conducted according to the Bergeys manual (conventional method) and based on morphological, physiological and biochemical analysis using API 50 CHL kit was also carried out. The results of identification and chemical test showed that 26 isolates were identified as LAB were 16 isolates (61.5%) with rod shape and 10 isolates (38.5%) with cocci shape. Based on proteolytic activity, the isolates composed of two bacteria species namely *Lactobacillus plantarum* (YN1.1, YN1.3, YN 1.8 and isolates YN 2.25). and the other bacteria was *Lactobacillus pentosus* (YN 1.6.). five isolates had proteolytic activity, where three of them had the best proteolytic activity namely *Lactobacillus plantarum* YN 1.1, *Lactobacillus plantarum* YN 1.3 and *Lactobacillus pentosus* YN 1.6 . All of these isolates had the ability to ferment goat milk which was indicated by the increase of bacteria viability reaching up to $10^9$ cfu/ml and also decreaced of pH value at the final stage of fermentation. It can be concluded that isolated LAB from spontaneous fermented local goat milk could be used as starter culture and could be inproved as commercial product.

**Keywords:** identification, API 50 CHL,*Lactobacillus plantarum*, *Lactobacillus pentosus*, viability, pH.

### 1. Introduction

Lactic acid Bacteria (LAB) is an important bacteria in fermented products, it is functioned both in fermentation process or increasing nutrient value of fermented products. LAB is a gram-positive, non sporing, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidious, acid tolerant and strictly fermentation (Khalid 2011). Generally LAB widely distributed in nature and found as indigenous microflora in raw milk and fermented milk with spontaneous fermentation. LAB mostly dominant in milk poduct with different species and also detected in human (Martin et al 2003) and also exist in animal (Fujisawa and Mitsuoka 1996). LAB have been extensively used in food fermentation, including the production of dairy products, and its proteolitic activity is very important in producing flavor compounds of end product (Moulay et al 2013). LAB are generally associated with habitats which are rich in nutrients, such as various food products (milk, meat, vegetables), and are important in dairy products. Fermented dairy products have been major part of the diet of people around the world, where LAB are used in making starter culture and proteolytic systems play an essential in nitrogen metabolism of LAB in milk (Moulay et al 2006). The Study by Moulay et al (2006); Anas et al (2010) and Moulay et al (2013) noted that many kinds of LAB species will be found when isolation of LAB from goat milk and goat milk products were carried out. *Lactobacillus plantarum* and *Lactobacillus fermentum* have been reported to be the most commonly associated LAB species with spontaneous lactic acid fermentation .

In Indonesia, fermented products using goat milk as basic material is not popular among consumers, and the consumers preferred to consume cow milk and its fermented products. This condition was due to the smell of goat milk is stronger and they do not like the taste compared to cow milk. Therefore by fermentation of goat milk, it is expected the fermented product could increase consumers likeness.

In general LAB used in fermentation of milk products are proteolytic and caused coagulation of milk due to the instability of milk protein, and this curd could change the texture of milk products. Proteolytic system of LAB is important for the growth of microorganisms and in native free amino acid of milk coresponding to 8-16 % of maximum found in coagulated milk (Kok 1990). Proteolytic system involved in casein utilization within LAB cells and give contribution to the development of organoleptic properties of fermented milk products (Yamina et al 2013; Moulay et al 2006).

The aim of this study was to isolate LAB from spontaneous fermented local goat milk and to classify those microorganisms based on the morphological and biochemical characterisistics and also to determine their proteolytic activity and the ability as starter culture in fermentation of local goat milk.

## 2. **Materials and Methods**

2.1. Sample preparation and isolation procedure.

Goat milk from local type breed (Ettawa filial breed) as samples were collected from households in Ngaglik village, the regency of Sleman, Yogyakarta. Samples were taken after milking of goat and collected using sterile bottles and delivered to Dairy Microbiology Laboratory of Interuniversity Food and Nutrition Centre (PAU) Gadjah Mada University, Yogyakarta, and closed tightly and fermented spontaneously for 4 days till the pH value was decreasing to 4.3.

Medium used was MRS (*de Man Ragosa Sharpe code CM 359*) added with 1% $CaCO_3$ as selective medium. Sampling for isolation was done after finishing spontaneous fermentation by taking 10 ml of fermented goat milk, and then it was put into 90 ml sterilized-diluent solution containing 1% of water peptone and the process of dilution till $10^6$ cfu/ml was obtained. Each dilution was taken 1 ml for pour-plated at MRS medium in order to be able to add 1% $CaCO_3$, and further was incubated aerobically at $36^o$ C for 48 hours. To prevent the growth of fungi, 1% cycloheximide was added into medium (Beukes et al 2001).

The colony which was considered as LAB would formed a clear zone in surrounding of colony growth. After the process of planting in MRS medium containing $CaCO_3$, and incubated at $37^oC$ for 48 hours. Furthermore when the colonies were growing and formed clear zone, then it was purified by making scratch using streak plate method and it was done 3 times to ensure its purification. Before further testing, the isolates which were considered as already purified was stored in medium containing 10% skim milk and 20% glycerol as isolate stock at freezing temperature (- $20^o$ C).

2.2. Identification of Lactic Acid Bacteria

Identification of each colony which was considered as selected LAB was conducted by conventional method based on morphological, biochemical and physiological characteristics of isolate. The morphologically test based on the formation of colony, size, color, edge form and texture of colony, then the selected colony was stained Gram to find out whether Gram negative or positive and continued by looking the formation of cell using 1000-magnified microscope. Profile matching method based on Bergey's manual of Systematic Bacteriology with some modification were used for characterization and identification of LAB isolates (Wang et al 2008).

2.3. Isolation of Lactic Acid Bacteria Based on Proteolitic Characteristic**.**

The one which was selected as LAB were then tested for the proteolytic activity. From the previous testing results, it was then continued with determining the proteolytic activity by growing the selected isolate in 2% skim medium agar and incubated at $37^o$ C for 48 hours and measured the amount of clear zone formed around the colonies ( Walter,1984). Bacteria stated positive had proteolytic activity will be purified and stored for the next test

2.4. Physiological Testing.

Physiological testing conducted also include the influence of temperature and pH toward the growth of isolated LAB and the growing temperatures treatment were $15^o$ C, $25^o$ C, $40^o$ C and $45^o$ C respectively, while for pH values used were 3.0 ;4.0; 6.0 and 7.0 respectively. These tests were conducted according to the method as described by Hammes and Vogel (1995) and Ray (1996) with a slight modification, and the aim of this test was to determine whether the isolated LAB could be classified into psycrophylic, mesophylic or thermophylic group.

2.5. Biochemical Fermentation Of Isolate

Biochemical testing conducted include the analysis of dextran and acid formation from various types of sugar. Fermentation capabilities of selected isolates using 49 types of carbohydrates contained in the API 50 CHL kit, and the tests were carried out to determine the biochemical properties of isolates within fermented carbohydrates. The ability of isolates to ferment carbohydrates indicated by a color change of the basal medium used, where the initial basal media color was purple and the positive isolates changed the fermentation medium turn to yellow basal medium. Isolates were identified (genus and species) using the API 50 CHL system (Bio-merieux, Japan) ( Bukola et al 2008; Maqsood et al 2013).

2.6. The isolate fermentation capability as starter culture.

The fermented capability of isolates was conducted by inoculating of each isolate culture which had best proteolityc activity into 8% sterile skim milk and incubated at 37 $^o$C for 16 hours and viability of LAB were enumerated during fermentation process. Viability of bacteria during fermentation measurement through test namely TPC (Total Plate Count), where sample was taken every 4 hours as described by Dave and Shah (1998), with sligh modification. The pH value of LAB was measured using pH meter according to the method as described in AOAC (2000).

## 3. **Results and Discussion**

3.1. Characterized of Isolate

The results of isolation of spontaneus fermented goat milk using MRS and $CaCO_3$ media had showed that out of 60 positive colonies on media produced acid. Further isolation resulted 26 different isolates which were

identified as LAB, and the identification was started by looking at the shape of cell from each colony and followed by Gram staining, catalase testing and then determined its proteolytic activity. Results of this examination indicated that these 26 isolates were considered as LAB which was characterized by positive Gram, negative catalase, not motile and able to live in microaerophylic condition. Early identification were also carried out by looking the characteristics of LAB obtained and it was observed that rod shape LAB were as many as 16 isolates (61.5 %) and coccus-shaped were showed by 10 isolates (38.5 %). Four isolates of 16 isolates were rod chain shape, while coccus chain shape as much as 3 isolates of 10 coccus shape isolates, and in general the dominant LAB were those rod shaped (Table 1).

Tserovsk et al (2004) reported that LAB species isolated from goat milk were rod and cocci shaped either chained or clustered, and characterized by positive Gram, negative catalase, microaerophylic, resistant to acid, not producing spore, and producing lactate acid as fermentation product. Furthermore, Moulay et al (2013) had isolated microorganisms from goat milk and found that 13 isolates of LAB were from 138 total isolates. From these 13 isolates of LAB there were 8 coccus shaped isolates and 5 other isolates were rod shaped. Other researches such as Abdelgadir et al (2001) and Savadogo et al (2004) had also identified the isolation from fermented cow and lamb milk and observed that the most dominant bacteria were those from genus *Lactobacillus.* A similar observation was reported by Khalid (2011) who also noted that LAB were positive Gram bacteria without spore, with negative catalase, microaerophylic, resistant to acid, and can do fermentation. Holzapfel et al (2001), also found that LAB were bacteria in rod or coccus shapes, with negative catalase, not motile, homo fermentative or hetero fermentative, and growing in low acid condition

Table 1. The results of culture isolated in MRS media containing $CaCO_3$
(Cell shape. Gram, catalase,colour and clear zone of isolates)

| Isolate | Catalase | Gram | Cell form | Colour of colony | Clear zone on (**) Skim milk 2% |
|---|---|---|---|---|---|
| YN 1.1 | - | + | rod | cream | +++ |
| YN 1.2 | - | + | rod | white | - |
| YN 1.3 | - | + | rod | white | +++ |
| YN 1.4 | - | + | rod chain | white | - |
| YN 1.5 | - | + | rod | white | - |
| YN 1.6 | - | + | rod | cream | +++ |
| YN 1.7 | - | + | rod | white | - |
| YN 1.8 | - | + | rod | white | ++ |
| YN 1.9 | - | + | coccus | white | - |
| YN 1.10 | - | + | coccus | white | - |
| YN 1.11 | - | + | rod | white | - |
| YN 1.12 | - | + | rod | cream | - |
| YN 1.13 | - | + | rod | cream | - |
| YN 1.14 | - | + | coccus | cream | - |
| YN 1.15 | - | + | coccus | white | - |
| YN 1.16 | - | + | coccus chain | white | - |
| YN 1.17 | - | + | coccus chain | white | - |
| YN 1.18 | - | + | rod chain | white | - |
| YN 2.19 | - | + | rod chain | white | - |
| YN 2.20 | - | + | coccus chain | cream | - |
| YN 2.21 | - | + | rod chain | white | - |
| YN 2.22 | - | + | coccus chain | white | - |
| YN 2.23 | - | + | coccus | white | - |
| YN 2.24 | - | + | rod | white | - |
| YN 2.25 | - | + | rod | white | ++ |
| YN 2.26 | - | + | rod | white | - |

(-) Negative reaction, (+) Positive reaction
(-)** Negative activity , (+)**Positive activity, (++)**Positive activity (+++) ** stronger activity.

While Axelson (2004) determined that LAB were bacteria with morphological, physiological and metabolical characteristics belong to positive Gram group, with negative catalase, coccus or round shaped, without spore, not

motile, without respiration, but can grow on aerobic or microaerotolerant conditions, and can produce lactate acid as main metabolic product from carbohydrate fermentation.
Isolates which were estimated as LAB were continued determined for their proteolytic activity by growing the selected isolates in 2 % skim milk media. Skim milk media were inoculated with the selected isolates and incubated at $37^0$C for 24 hours and 48 hours. After incubation, clear zone was measured and the results of the isolation of LAB and the proteolytic activity were presented in Table 1.

The results of proteolytic activity determination showed that there were some clear zones around the colonies in each isolate and from inoculated 26 isolates of LAB into 2 % skim milk media agar, 5 isolates were observed had the ability to hydrolyze protein and it is shown by the emergence of clear zones around the colony. It is interesting to note that of 5 isolates (YN 1.1, YN 1.3, YN 1.6, YN 1.8 and YN 2.25) producing clear zone only 3 isolates (YN 1.1, YN 1.3, and YN 1.6 ) had stronger proteolytic nature as shown by greater clear zone than other isolates. All isolates with big proteolytic activity were those in rod shape. Clear zone in the skim milk media was produced by the selected isolates after incubation for 24 hours and 48 hours. Every isolate producing clear zone in skim milk media indicated that this isolate could degrade protein or had proteolytic activity. Clear zone was measured using digital caliver and the measurements were tabulated as shown in Table.2

Table 2 : Measurement of clear zone of isolat (YN 1.1, YN 1.3, YN1.6 YN. 1.8 dan YN 2.25) on skim milk media

| Isolate Code | Clear zone average(mm) | |
|---|---|---|
| | 24 hours | 48 hours |
| YN 1.1 | 16*** | 19*** |
| YN 1.3 | 16*** | 20*** |
| YN 1.6 | 18*** | 23*** |
| YN 1.8 | 12** | 14** |
| YN 2.25 | 14** | 15** |

( *** ) bigger clear zone , (** ) smallest clear zone

Data in Table 2 showed that every isolate had different proteolytic activity. Isolate YN 1.6 had 18 mm after 24 hours incubation and increase to 23 mm after 48 hours incubation. Clear zone on isolate YN1.6 indicated this isolate had a bigger proteolytic activity than other isolates. While Isolate YN 1.1 as well as Isolate YN 1.3 had same clear zone size (16 mm) after incubation for 24 hours. Proteolytic activity of Isolate YN 1.8 was indicated by 12 mm clear zone and this isolate had the smallest proteolytic activity compared to other isolates, while Isolate YN 2.25 had a better proteolytic activity than Isolate YN 1.8 and proteolytic activity of the isolate YN 1.3 was shown in Figure 1.
Proteolytic activity of all isolates produced in this study were almost similar or equal to the results reported by Yamina et al (2013), where proteolytic activity of fermented camel milk indicated the presence high proteolytic activity area which was more than 20mm in clear zone diameter on the first group contained 10 strains ( 1,1,11,13,19,20,22-25), strain with medium proteolytic activity whose diameter was between 10 and 15 mm were the second group contained 11 strains ( 3-6,8,10,12,16,18 and 21), and for group with low proteolityc activity area whose diameter was less than 10 mm contained 4 strains ( 7,9,14 and 15 ).
Seifu et al (2002) noted that LAB which derived from fermented milk isolation and had proteolytic activity were *Lactobacillus plantarum, Lactobacillus delbrueckii subspecies bulgaricus, Lactobacillus* salivarius, *Lactococcus lactis subspecies lactis, Lactococcus lactis subspecies cremoris, and Enterococcus faecalis*. The presence of proteolytic activity was signified by the reduction of pH value in skim milk media during fermentation process.

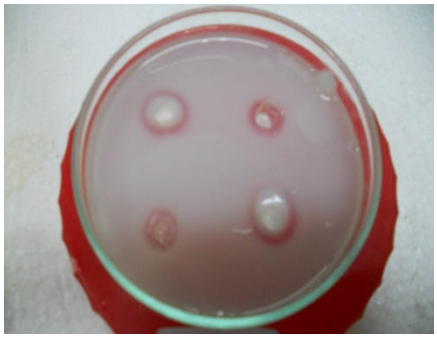

Figure 1. Proteolytic activity of isolates *L.plantarum* YN 1.3 after 24 hours incubation at $37^o$ C

As mentioned by Omafuvbe and Enyioha (2011), the result of isolation from commercial yogurt showed that 13 % isolates had nature proteolytic activity and *Lactobacillus acidophilus* had the greatest proteolytic activity than all isolates. Isolates with proteolytic activity were tested to determine their physiological and biochemical characteristics, and it allows to sort out the species with isolates from which the expected proteolytic was obtained.

3.2. Physiological characteristics of proteolytic isolates

Physiological test involved examining the influence of temperature and pH on the growth of LAB as the result of isolation. The examination of the influence of temperature was aimed to understand the type of bacteria as the result of isolation, whether it belongs to psycrophylic, mesophylic or thermophylic groups. Psycrophylic microbes grow at temperature less than $15^0$C and optimum at $20^0$C. Mesophylic group can grow at warmer temperature, ranging from $15^0$C-$45^0$C, while thermophylic group can grow at high temperature, which is between 45 and $80^0$C. Cellular growth was indicated by turbidity after incubated for 48 hours on MRS media. The results of morphological and physiological tests over five isolates were shown in the Table 3.

The results of identification which are shown in Table 3 were LAB YN 1.1; YN 1.3; YN 1.6; YN 1.8 and YN 2.25, which also had proteolytic activity and able to grow at temperature in the range of $20^o$ to $40^o$ C, where LAB YN 1.1 and LAB YN I.3 were able to grow at 45 $^0$ C. All isolates were not able to grow at 50 $^o$C. The result indicated that these LAB were mesophylic bacteria and isolate YN 1.1 and YN 1.3 also had the thermophylic characters. While the influence of pH showed that all isolates could grow in low pH value (3.0) and also grow well at above pH 7.0 such as at pH 8.5. The results for pH tests indicated that LAB YN 1.1, YN 1.3, YN 1.6, YN 1.8 and YN 2.25, could grow well at low pH and also at higher pH value. Moulay et al (2013) had found that LAB isolated from goat milk had the ability to grow at $30^0$C, but not all isolates could grow well at $45^0$C. LAB isolates had proteolytic ability and could grow at low pH and produced lactic acid. Badis et al (2004) reported that 6 isolates which was isolated from goat milk were belong to genus *Lactobacillus* with mesophylic characteristic (SH10, SH12, SH13) and also with thermophylic characteristic (SH8, SH9 and SH11). Carr et al (2002) declared that LAB were firstly isolated from milk, and it is then isolated from fermentation products such as milk, meat, vegetable, drink and bread. Naturally, LAB can be obtained from fermentation products (Caplice and Fitzgerald, 1999). Furthermore, Savijoki et al (2006) explained that LAB used in manufacturing processes of fermented foods and the most important application is their use as starter strains in manufacture of various fermented dairy products.

3.3. Capability of carbohidrate fermentation (API 50 HCL KIT.)

The capability of fermentation among the selected isolates was determined using 49 kinds carbohydrate contained within API 50 CHL Kit. The test was conducted to determine the biochemical characteristic of isolates in fermenting carbohydrate. The capability of isolate in fermenting carbohydrate was shown by the discoloration of basal medium, where the early color of this basal medium was purple. Isolates with fermentation will change the color into yellow, and the results of testing using API 50 CHL Kit is shown in Table.3.

The capability of isolates to ferment carbohydrate in API 50 CHL Kit was observed during fermentation, and it was found that not all carbohydrates could be fermented by the selected isolates of LAB. This condition was seen during discoloration, where some of bacteria could not do the fermentation perfectly as shown by the absence of yellow color in substrate or basal solution. The change only occured when the basal solution changed its color from purple to green (Table 3 code with V ). This was posibly due to the lack of capability of enzyme produced by isolate to decompose sugar in the basal solution. LAB with high fermentation activity will change the basal solution's color from purple to yellow, and LAB isolates without fermentation capacity would not changing the color of basal solution at all.

Phenotypic data include morphological, physiological and biochemical characterization and the data were collected by probabilistic identification using API Web Database V51. The Results indicated that isolates could be classified as follows: first isolate (YN 1.1) was *Lactobacillus plantarum* with 89 % identical rate and second isolate (YN 1.3) was *Lactobacillus plantarum* with 99.9 % identical rate. While third isolate (YN 1.6) was *Lactobacillus pentosus* with 97.3 % identical rate, fourth isolate (YN 1.8) was *Lactobacillus plantarum* with 99.9 % identical rate and fifth isolate (YN 2.25) was *Lactobacillus plantarum* with 69.9 % identical rate.

The Results of identification using API 50 CHL KIT and the determination of phenotypic rate using conventional method were showing that almost all selected isolates were LAB namely *Lactobacillus plantarum* and only one assumed that isolate was *Lactobacillus pentosus*.

Table 3: Phenotype characteristics of Lactic Acid Bacteria Isolated from Spontaneus fermented goat milk and profile fermentation of sugar.

| Characteristic | Isolate | | | | |
|---|---|---|---|---|---|
| | YN 1.1 | YN 1.3 | YN 1.6 | YN 1.8 | YN 2.25 |
| Cell shape | R | R | R | R | R |
| Gram staining | + | + | + | + | + |
| Gas produced | - | - | - | + | - |
| Catalase | - | - | - | - | - |
| Motility | - | - | - | - | - |
| Dextran | - | - | - | - | - |
| Growth at | | | | | |
| $15^0$ | Nt | Nt | Nt | Nt | Nt |
| $20^0$ | + | + | + | + | + |
| $30^0$ | + | + | + | + | + |
| $45^0$ | + | + | + | + | + |
| $50^0$ | - | + | - | - | - |
| NaCL 6.5 % | + | + | + | + | + |
| pH 3.5 | + | + | + | + | + |
| pH 4.5 | + | + | + | + | + |
| pH 5.5 | + | + | + | + | + |
| pH 8.5 | + | + | + | + | + |
| pH 9.6 | - | - | - | - | - |
| DAP tipe | Nt | Nt | Nt | Nt | Nt |
| **Tipe fermentation:** | | | | | |
| Gliserol | V | V | - | - | - |
| Erythritol | - | - | - | - | - |
| D-arabinosa | - | - | - | - | - |
| L-arabinosa | + | + | + | + | + |
| D-ribose | + | + | + | + | + |
| D-xYNose | - | - | + | _ | + |
| L-XYNose | - | - | - | - | - |
| Adonitol | - | - | - | - | - |
| Methyl Xylopyranoside | - | - | - | - | - |
| D-galactose | + | + | + | + | + |
| D-glukose | + | + | + | + | + |
| D- fructose | + | + | + | + | + |
| D-mannose | + | + | + | + | + |
| L-Sorbose | + | + | + | + | + |
| L-rhamnose | V | - | - | V | - |
| Dulcitol | - | - | - | - | - |
| Inocitol | - | - | - | - | - |
| D-mannitol | V | - | - | - | - |
| D-sorbitol | - | + | + | + | + |
| Metyl-αD mannopyranoside | - | + | + | - | - |
| Metyl- αD Glucopyranoside | - | + | - | + | - |
| N-acetyl glucosamine | - | - | - | - | - |
| Amygdaline | + | + | + | + | + |
| Arbutine | + | + | + | + | + |
| Esculine | - | - | - | - | - |
| Salicine | + | + | + | + | + |

Table 3: Phenotype characteristics of Lactic Acid Bacteria Isolated from Spontaneus fermented goat milk and profile fermentation of sugar.

| Characteristic | Isolate | | | | |
|---|---|---|---|---|---|
| | YN 1.1 | YN 1.3 | YN 1.6 | YN 1.8 | YN 2.25 |
| D-cellobiose | + | + | + | + | + |
| D-maltose | + | + | + | + | + |
| D-lactose | + | + | + | + | + |
| D-melibiose | + | + | + | + | + |
| D-sacharose | + | + | + | + | + |
| D-tetralose | + | + | + | + | + |
| Inulin | V | V | V | - | - |
| D-melezitose | + | + | + | + | + |
| D-raffinose | + | + | + | + | + |
| Amidon | + | + | + | + | - |
| Glycogene | - | - | - | - | - |
| ZYNitol | - | - | - | - | |
| Gentibiosa | V | V | V | + | V |
| D-Turanose | + | - | + | + | + |
| D-Lyxose | - | - | - | - | - |
| D-Tagadose | + | V | - | - | - |
| D-Fucose | - | - | - | - | - |
| L-Fucose | - | - | - | - | - |
| D-Arabitol | - | - | - | - | - |
| L-Arabitol | - | - | - | - | - |
| Potasium gluconat | V | + | V | + | - |
| Pot-celogluconat | - | - | - | - | - |
| Pot-2 celogluconat | - | - | - | - | - |
| Species | *Lactobacillus. plantarum* | *Lactobacillus plantarum* | *Lactobacillus. pentosus* | *Lactobacillus. plantarum* | *Lactobacillus. Plantarum* |

(+) positive reaction, (-) negative reaction.
(R ) rod, (G+).gram stain positive reaction, (Nd) not detected

Anas et al (2012) had reported that the isolation of LAB from fresh milk of Alger Goat was identified as *Lactobacillus plantarum* and *Lactobacillus rhamnosus*. Mathara et al (2008) had found that *Lactobacillus plantarum* was the most dominant species among LAB strains isolated from traditional fermentation milk in Kenya. It means that *Lactobacillus plantarum* isolated from Maasai fermentation milk had the ability as probiotic bacteria. Futhermore Mathara et al (2004) noted that *Lactobacillus plantarum* was an important species in some fermentation products from vegetable or milk product. Some strains were already known as able to produce anti-microbial substance. Kaktcham et al (2012) declared that the isolation of LAB from Cameron traditional fermentation drink isolates were *Lactobacillus plantarum* (62 %), *Lactobacillus rhamnosus* (24 %), and *Lactobacillus fermentum* (10%) respectively.

3.4. Capability of each isolates as starter culture to ferment milk

Capability of isolate as starter culture to ferment milk was carried out by inoculation of each isolate on skim milk ( 8%,) and incubated at $37^0$C for 16 hours. To identifify the capability of isolate to ferment was carried out by measuring the pH value and viability of LAB during fermentation process. The isolate were used to measure the capability as starter culture was isolate with the biggest proteolityc activity namely YN 1.1, YN 1.3 and YN 1.6 respectively.

**3.4.1.** pH values

The capability of each isolate to decrease the pH value in milk was measured at the early fermentation stage until the end of fermentation process. The decreased pH value was measured for each isolate used during fermentation, and the alteration of pH during fermentation process were shown in Figure 2.

At the early stage of fermentation process, the pH value of each milk was varied in the range of 6.64 to 6.70 and decreasing to the range of 4.72 – 4.42 at the end of process. The pH value of starter prepared using *Lactobacillus pentosus* YN 1.6 showed the biggest change from 6.72 to 4.42 while pH of *Lactobacillus plantarum* YN 1.3 was decreased from 6.56 to 4.48 and the smallest change was in milk using *Lactobacillus plantarum* YN 1.1.from 6.65 to 4.72. at the end of fermentation process.

The statistical analysis showed there were significant differences (P<0.05) between isolates at the end of fermentation process. It is believed that pH values reduction due to milk sugar hydrolisis into lactic acid, and beside lactic acid other acids such as acetic acid, propionic acid and butyric acid were also produced

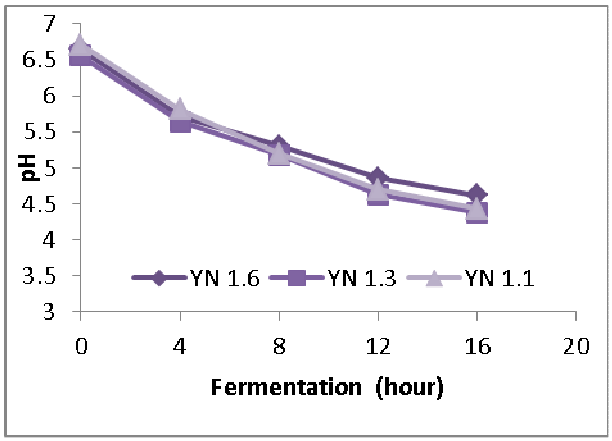


Figure 2. pH value during fermentation process using *Lactobacillus plantarum* YN 1.1, *Lactobacillus plantarum* YN 1.3 and *Lactobacillus pentosus* YN 1.6

Seelee et al (2009) reported that pH value decreased during the milk fermentation and the longer fermentation time the lower the pH value will be. Piano et al. (2006) also noted that the change of pH value was related to viability of microorganism in milk fermentation.

3.4.2. Viability of Lactic Acid Bacteria**.**

The growth pattern of LAB during fermentation process was presented in Figure 3.

Viable bacterial count of sample during fermentation process was conducted every 4 hours as long as 16 hours fermentation of milk. The viability of LAB, increased after 4 hours of fermentation and at the early stage of fermentation the growth of LAB on each isolate used was in the range of $10^6$ cfu/ml, and at the end of fermentation LAB was increased to $10^9$ cfu/ml. Viability of LAB was increased as the fermentation time was also increased. The end of fermentation process was characterized by formation of curd in milk used, where this was due to the coagulation of milk protein. At the end of fermentation process (16 $^{th}$ hours) there was a slightly difference in viable of LAB number with *Lactobacillus pentosus* YN 1.6. (7.6 x $10^9$ cfu/ml) compared to the one using *Lactobacillus plantarum* YN 1.1. (3.4 x $10^9$ cfu/ml) or with *Lactobacillus plantarum*. YN 1.3 (.7.3 x$10^9$ cfu/ml).

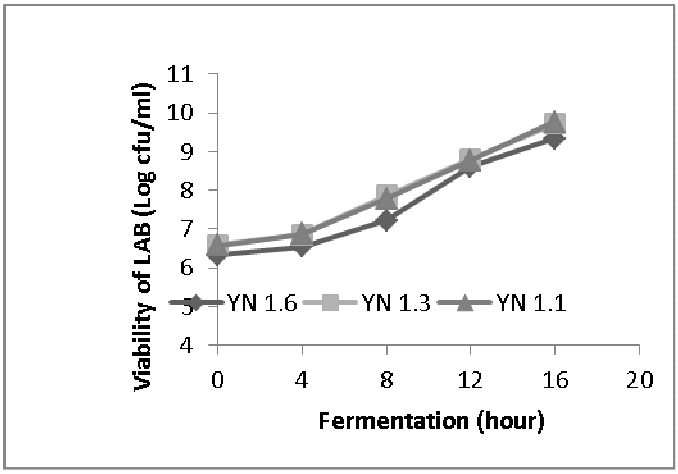


Figure 3 : LAB growth pattern during fermentation process of bacteria *Lactobacillus plantarum* YN 1.1., *Lactobacillus plantarum* YN 1.3 and *Lactobacillus pentosus* YN 1.6

The statistical analysis showed there were significant differences ($P<0.05$) viability of LAB between isolates at the end of fermentation process. Overall LAB isolates YN 1.1, YN 1.3 and YN 1.6 were able to ferment milk, which was indicated by the increased count of viable of LAB during fermentation and formation of curd in milk used at the end of fermentation. Antara et al (2002) reported that the number of LAB, during fermentation process (5 days), was rapidly increased from initial count of $1.72x10^5$ to $8.4x10^8$ cfu/g. Parente et al ( 1997) noted that LAB were used widely in fermented foods as starter cultures especially in dairy products, while Kok (1990) noted that proteolytic system of LAB was nescessary for rapid growth of these organisms in protein rich media hence a sufficient concentration of free amino acids could affected the cell density. Futheremore, Martin and Chou (1992), noted that candidate of LAB for use as dietary adjunt and exert has influence and it must conform to certain requirement and that viable bacteria count $10^8$ -$10^9$, generally recognised necessary at the time of consumption.

## 4. Conclusion

- The results of this study concluded that the isolation and identification of the isolate of LAB from spontaneous fermented local goat milk had shown its proteolytic activity.
- These isolates composed of two bacteria species, namely *Lactobacillus plantarum* (YN1.1, YN1.3, YN 1.8 and isolates YN 2.25) and the other bacteria was *Lactobacillus pentosus* ( YN 1.6.)
- Proteolityc bacteria isolates Lactobacillus plantarum (YN1.1, YN 1.3 and YN 1.6) had the ability to ferment milk and could be used as starter culture to produce fermented milk and also could be used commercially.

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