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Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk

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Abstract

A total of 83 lactic acid bacteria isolates belonged to six genera Lactobacillus (26.51%), *Lactococcus* (21.69%), *Leuconostocs* (18.07%), *Streptococcus* (9.64%), *Pediococcus* (12.05%) and *Entrococcus* (9.64%) were identified. *Streptococcus, Leuconostoc, Entrococcus, Pediococcus* and *Lactococcus* were cocci and Lactobacillus was rod shaped. In the present study, the dominant genera of lactic acid bacteria identified from raw cow milk samples were *Lactobacillus* (26.51%). The study was successful in isolating and identifying the naturally occurring LAB from raw cow milk. From this we can conclude that raw cow milk is a good source of lactic acid bacteria.

Keywords: Cow Milk, Identification, Isolation, Lactic Acid Bacteria.

Introduction

Cow Milk is a pale liquid produced by the mammary glands of cow. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. It contains many other nutrients including protein and lactose (Pehrsson et al., 2000). Moreover, Milk itself is known as one of the natural habitats of lactic acid bacteria (LAB) (Delavenne et al., 2012), (Wouters et al., 2002).

Lactic acid bacteria (LAB) are a group of Gram-positive, non-sporulating, anaerobic or facultative aerobic cocci or rods, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrates (Axelsson., 2004), (Hayek and Ibrahim., 2013). Lactic acid bacteria (LAB) are naturally present in milk and milk products. LAB is generally associated with habitat rich in nutrients such as milk, cheese, meat, beverages and vegetables. In addition, (Tserovska et al., 2002), (Chen et al., 2005) showed that lactic acid bacteria could be also isolated from soil, lakes, intestinal tract of animals and humans. Lactic acid bacteria (LAB) have been used for the fermentation of food and feed products since ancient days and today their major applications are still in the food and feed industry as starter cultures (Desmons et al., 1998), (Boonmee et al., 2003).

Lactic acid bacteria are the most important bacteria in desirable food fermentations, being responsible for the fermentation of sour dough bread, fermented foods and beverages, all fermented milks and fermented vegetables. It plays an essential role in the production of all dairy products and is involved in the production of many other fermented foods and beverages, sausages, pickles, boza etc. Based on the end product of glucose fermentation lactic acid bacteria are grouped as either *Homofermenters or Heterofermenters.* The *Homofermenters* produce lactic

acid as the major product of fermentation of glucose. The *Heterofermenters* produce lactic acid, carbon dioxide, acetic acid, and ethanol from the fermentation of glucose. According to (Sharma et al., 2012), (Steele et al., 2013) LAB are recognized for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits.

Many LAB species play an important role in the ripening process of cheese, especially to improve the consistency, aroma and flavor (Hannon et al., 2003), (Duan et al., 2008). Certain LAB strain characterized by their ability to transform lactose and improves the digestibility of fermented dairy products (Weinberg et al., 2007) as well as their preservation (Abdelbasset and Djamila., 2008). They also employed for improvement of the taste, texture and viscosity in the manufacture of dairy products (Soukoulis et al., 2007). Lactic acid bacteria can be recovered from fermented foods and beverages, vegetables, milk and milk products. The objective of the study was to isolate and identify naturally occurring lactic acid bacteria from raw cow milk.

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Materials and Methods

Sample collection

The study was conducted from February to April 2016 to isolate and identify the naturally occurring lactic acid bacteria from raw cow milk. A total of Twenty Five raw cow milk samples were collected from lactating cows in the surrounding area of the city Debrezeit, Ethiopia. Samples were collected using sterilized sample bottles and brought to laboratory with icebox for microbiological investigation. Samples were kept in a refrigerator (around 4°C) till the analysis begins.

Isolation of lactic acid bacteria

For isolation of lactic acid bacteria, 10 milliliters of each milk samples were homogenized with sterilized peptone physiological saline solution (1% peptone (Oxoid), 0.9% NaCl) for about 1-3 minutes aseptically. Appropriate serial dilution (10^{-1} to 10^{-6}) was prepared for each sample using 1mililiters of homogenate. A volume of 0.1 milliliters of appropriate dilutions was spread plated on MRS (OXOID) agar media. Then the plates were incubated for 48hours in anaerobic jar at 32^{o}C. Typical LAB characteristics colonies were randomly picked up and purify by streaking two or three times on fresh MRS agar plates followed by macroscopic and microscopic examinations. The colonies displaying the general characteristics of **lactic acid** bacteria were chosen from each plate for physiological and biochemical test.

Identification of Lactic Acid Bacteria to the Genus Level

For identification of **lactic acid** bacteria, overnight cultures of each isolate in MRS broth (Oxoid) were used. All isolates were initially tested for Gram reaction, motility, catalase enzyme. Identification of LAB was done based on morphology, physiology and biochemical characteristic (Harrigan and MacCance., 1976), (Kimaryo et al., 2000). These preliminary tests make it possible to classify the isolates in to genus on the basis of characteristic and tests of identification mentioned by (Harrigan and MacCance., 1976), (Holzapfel and Schillinger., 1992).

Pure bacterial isolates were further tested for cell morphology, motility, gram reaction, catalase production, acid production from glucose and growth at 10^{0}C, 15 ^{0}C, and 45 ^{0}C according to the methods described by (Kebede et al., 2007). Cell morphology of cocci or rod shaped, non-motile, gram-positive, catalase-negative, isolates with characteristic were considered as lactic acid bacteria. Four isolates were gram negative and catalase positive which are not the characteristics of LAB. Isolated lactic acid bacteria were tested for fermentation of Glucose, Lactose, Xylose, Sucrose, Melibiose, Raffinose and Sorbitol (Holt et al., 1994). All LAB strains were characterized and identified to the genus level according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Results and Discussion

A total of 83 lactic acid bacteria isolates were characterized and identified to genus level from twenty five raw cow milk samples collected from the surrounding area of the city Debrezeit. Morphological, physiological and biochemical characteristics of isolated genus are shown in (Table1). All isolates were gram positive, catalase negative and non-motile. The cell morphology of all isolates was evaluated through microscopic observation and the majorities (N=61) were found to be cocci and the remaining (N=22) were rod shaped. By comparing the result of morphological, physiological and biochemical tests the isolates were grouped in to six genera of lactic acid bacteria. With this, isolates were grouped in to *Lactobacillus* (26.51%), *Lactococcus* (21.69%), *Leuconostocs*

(18.07%), *Streptococcus* (9.64%), *Pediococcus* (12.05%) and *Entrococcus* (9.64%). The number of isolates grouped in the identified genera of LAB showed in (Figure 1). LABs are bacteria in rod or coccus shapes, with negative catalase, not motile, homo fermentative or hetero fermentative and growing in low acid condition (Holzapfel et al., 2001). Lactic acid bacteria (LAB) are one of the microorganisms that dominate fermented food (Guasch et al., 2005), (Robert., 2008). Lactic acid bacteria (LAB) belong to a group of gram positive bacteria that produce lactic acid as their main fermentation product into the culture medium and generally recognized as safe (Konings., 2000).

Table 1- Morphological, physiological and biochemical characteristics of isolated genera of LAB

Tests	***Enterococcus***	***Lactobacillus***	***Leuconostocs***	***Streptococcus***	***Lactococcus***	***Pediococcus***
Cell morphology	Cocci/round	Rods	Cocci/ovoid	Cocci/chain	Cocci/round	Cocci/round
Gram test	+	+	+	+	+	+
Motility	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-
CO_2 from glucose	+	+	+	-	+	-
Fermentation of carbohydrate						
Glucose	-	+	+	+	+	-
Lactose	+	+	+	+	+	+
Xylose	+	±	±	±	±	+
Sucrose	+	+	±	±	-	+
Melibiose	±	±	-	-	-	-
Raffinose	+	-	-	-	-	±
Sorbitol	-	+	±	-	-	±
Growth at (T^o)						
10 ^oC	-	-	-	-	±	-
15 ^oC	+	+	+	-	+	+
45 ^oC	±	±	-	±	±	±
Growth at (%NaCl)						
2%	+	-	+	+	±	+
4%	+	±	-	+	+	+
6.5%	±	-	-	-	-	-

+= Positive, -= negative, ±= varies between isolates

Among the isolates only the genus *Lactobacillus* and *Lactococcus* were able to produce carbon dioxide from glucose fermentation. This showed that they are hetrofermentative LAB. Only some isolates in the genus lactococcus grew at10 ^oC. All isolates were able to grow in 2%NaCl except some difference in the genus *Lactococcus.* In contrast, none of the isolates grew in 6.5% NaCl. The ability of growth at 4%NaCl and at 45^oC was different among the isolates. However, all isolates in the genus *Leuconostocs* were not grow at 4%NaCl and at 45^oC. Positive growth at 10^oC was observed in some isolates of *Lactococcus* genera. In the pattern of carbohydrate fermentation all isolates showed positive result for glucose and lactose fermentation. However, there is difference in the fermentation of other carbohydrates. Among the isolates only *Entrococcus* and *Pediococcus* were positive for the fermentation of Raffinose. The genus *Lactococcus, Leuconostocs, Streptococcus, Pediococcus* were negative for Melibiose but, it was variable and strain dependent for enterococcus and lactobacillus genera. All 18 isolates in the genus *Lactococcus* were not able to ferment sucrose. But in other genera there is a difference in fermentation of sucrose.

Study by (Tserovska., 2004) showed 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 lactic acid as fermentation product. As LAB found in

nutrient rich substrates like milk, fermented vegetables, foods, beverages, it is possible to isolate and characterize them. It was proven that lactic acid produced by lactic acid bacteria not only keeps foods and beverages in the state of perfect preservation but can also promote the growth of healthy microbiota throughout the intestine beside of reducing the pathogens. LAB species are very important in food industry as beneficial organisms. One of the most important contributions of these microorganisms is the extended shelf life of the fermented products.

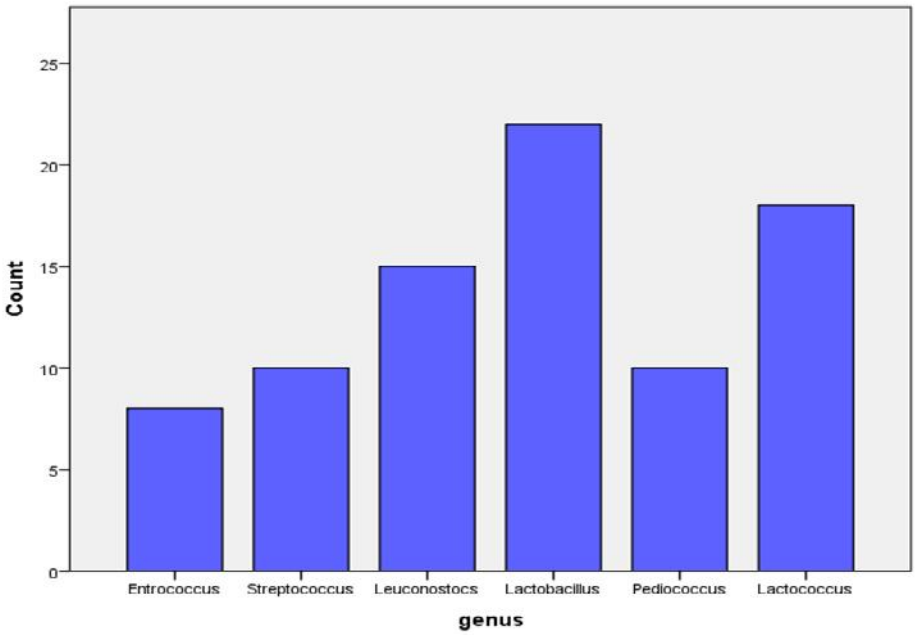

Figure 1. Number of isolates in identified genera of LAB

Isolation of LAB is also possible from other substrates like traditional fermented foods and beverages, sourdough. Generally, the lactic acid bacteria genus identified in the present study were comparable with other studies. Isolated genera of the present study were in agreement with (Vuysta and Vancanneyt., 2007) who reported that *Lactobacillus; Pediococcus, Streptococcus* and *Leuconstoc* were isolated from borde and shamita. In addition, (Hirayama and Rafter., 1999) indicated that L. fermentum, *Pediococcus spp, Leuconostoc spp, Enterococcus spp, C. humilis* and *S. exiguus* have been isolated from sourdough. In addition, (Sawsan et al., 2010) found *Lactobacillus, Lactococcus and Pediococcus* genera from raw cow milk, white cheese and rob in Sudan. In the present study, *Lactobacillus* (26.51%) was the dominant genera isolated from raw cow milk. The result was supported by (Badis et al., 2004b) who showed *Lb. helveticus, Lb. plantarum, Lb. delbrueckii subsp. bulgaricus, Lb. brevis* as the most abundant isolated species from raw goat's milk. Those isolated species were belonged to the genus *Lactobacillus.* In addition, (Mayeux et al., 1962) *Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Lactobacillus delbrueckii subsp lactis, Leuconostoc lactis* and *Leuconostoc citreum* were identified in South African traditional fermented milks.

The genera *Leuconostoc, Lactococcus* and *Lactobacillus* as a dominant isolate and other group included pyogenic *Streptococci* and enterococci also identified from Dhan traditional Butter (Guessas et al., 2012). Lactic acid bacteria produce lactic acid as their main end product of carbohydrate fermentation. LAB species are very important in food industry as beneficial organisms to be used as a starter culture. One of the most important contributions of these microorganisms is the extended shelf life of the fermented products.

The genera *Lactobacillus, Pediococcus* and *Lactococcus* belong to the lactic acid bacteria (LAB) and the strains of these genera are frequently used on a large scale in the production and preservation of many foods or as probiotics for human and animals (Holzapfel., 1998), (Osmanagaoglu., 2010). The present study had great success in isolating lactic acid bacteria from samples of raw cow milk. The result indicates that raw cow milk is a good source of lactic acid bacteria.

Conclusion

The study was conducted to isolate and identify the naturally occurring lactic acid bacteria from raw cow milk collected from lactating cows. With this a total of 83 lactic acid bacteria belonging to the genus *Lactobacillus, Lactococcus, Lecunostoc, Streptococcus, Enterococcus* and *Pediococcus* were identified from Twenty Five raw cow milk samples. The results obtained from the present study demonstrated that there is a diversity of lactic acid bacteria in raw cow's milk. The presence of LAB in milk and milk products enhances bioavailability of nutrients and act as a preservative.

From this study, we can conclude that raw can milk is a rich source of LAB. In addition, it was proven that raw cow milk contained both homofermentative and hetrofermentative lactic acid bacteria. This local raw milk could serve as source for beneficial lactic acid bacteria in future researches. Further studies will be done on the characterization, identification in to species level and their probiotic potential.

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