

RAW COW MILK AS A POTENTIAL SOURCE OF BENEFICIAL LACTIC ACID BACTERIA (LAB)

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ABSTRACT

Milk itself is also considered to be one of the nature habitats of the LAB, It contains the many other nutrients and including protein and lactose. The aim of the research was a isolation and identification of naturally occurring lactic acid bacteria from cow raw milk. A total of ninety isolates of lactic acid bacteria were collected of which seven genera belonging to *Lactobacillus* (29.32%), *Lactococcus* (23.68%), *Leuconostoc* (21.09%), *Streptococcus* (12.09%), *Aerococcus* (9.09%), *Pediococcus* (17.09%) and *Enterococcus* (12.09%) were identified. *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Lactococcus*, and *Aerococcus* were cocci and rod shape of *Lactobacillus*. In the current study, *Lactobacillus* (29.32%) was the main genera of lactic acid bacteria identified from cow raw milk samples. We can conclude from this research that raw cow milk is a good source of healthy lactic acid bacteria.

Keywords: Raw cow milk, Microorganisms, Lactic acid bacteria, Biochemical characteristics

INTRODUCTION

Milk products made from local raw milk tend to be a very important part of the daily diet, the essence of these products varies from region to region depending on the local area indigenous microflora, which in turn represents the climatic conditions of this area. These products have in one common feature: fermentation by lactic acid bacteria (LAB) is an essential part of this manufacture (Abd El Gawad, 2010). It is the main nutrient source for infant mammals are before they can digest other food forms. It contains the many other nutrients including protein and lactose (Pehrsson *et al.*, 2000). Milk itself is also considered to be one of the natural habitats of the LAB (Delavenne *et al.*, 2012 and Wouters *et al.*, 2002). The interest in foodborne micro-organisms is mainly due to the biotechnological potential of these new bacterial strains and species (Leisner *et al.*, 1999). Lactic acid bacteria (LAB) is a genus of gram-positive, facultative aerobic and anaerobic, non- sporulating, rods or cocci that generate this lactic acid as one of the carbohydrate metabolism's key fermentation products (Axelsson, 2004, Hayek and Ibrahim, 2013). There are naturally occurring lactic acid bacteria (LAB). There are various types of traditional milk products in our country that are made from goat and sheep milk such as, kashk, yogurt, cheese, gharaghooroot, etc. The composition of lactic acid bacteria in these products is more complex and inconstant compared with commercial organisms (Azadnia, 2009). They also worked to improve the taste, viscosity, and texture of dairy products manufacturing (Soukoulis *et al.*, 2007). Lactic acid bacteria (LAB) have been used in the fermentation of this feed and food products. since ancient times and are still mainly used in the food and feed industries today as starter cultures (Desmons *et al.*, 1998; Boonmee *et al.*, 2003). Fermentation of sourdough bread, beverages and fermented foods, all fermented vegetables, and fermented milk. Lactic acid bacteria are most of the important bacteria in beneficial food fermentations. This plays an important role in the manufacturing of all dairy products and includes the processing of many other beverages, pickles, sausages, boza, and fermented food, etc. The lactic acid bacteria are classified as either heterofermenters or homofermenters based on the result of glucose fermentation. The homofermenters produce glucose fermentation from lactic acid, acetic acid, ethanol, and carbon dioxide. According to (Sharma *et al.*, 2012; Steele *et al.*, 2013) LAB is known for its fermentation ability and therefore enhances food protection, enhances organoleptic properties, enriches nutrients, and raises health benefits. LAB may be extracted from fermented foods and vegetables, beverages, milk, and milk products. This research aimed to isolate and identify lactic acid bacteria from raw milk.

MATERIAL AND METHODS

Sample Collection

The research was performed between June to October 2019. To isolate and identify naturally occurring lactic acid bacteria from non - pasteurized cow milk. Thirty cow raw milk samples were collected from Jersey lactating cow in Annamalai Nagar of the Chidambaram city Cuddalore. These samples were collected by using the sterile sample bottles for microbiological investigation and

carried to the laboratory with an icebox. Samples were placed in about 4° C until the research had begun.

Isolation of lactic acid bacteria from raw milk

For lactic acid bacteria isolation, 10 mL of each milk sample is homogenized for around 1-3 minutes aseptically with sterilized peptone, and physiological saline solution (0.9 percent NaCl) and 1 percent peptone. Appropriate serial dilutions (10^{-1} to 10^{-7}) for each sample was prepared using 1mL of homogeneous milk. On MRS (OXOID) agar media, a volume of 0.1 mL of appropriate dilutions were spread plated. The plates were then incubated in an anaerobic jar at 32° C for 48 hours. Typical colonies of LAB characteristics are randomly picked up and cleaned by streaking on fresh MRS agar plates two or three times, followed by microscopic and macroscopic examinations. For physiological and biochemical testing different displaying colonies were selected from each plate and general characteristics of the lactic acid bacteria were studied

Identification of Lactic Acid Bacteria to the Genus Level

Overnight cultures of each bacterial isolates were used in MRS broth (Oxoid) to classify the lactic acid bacteria. Initially, all isolates were tested for Gram's reaction, enzyme catalase, motility. LAB was identified using physiology, morphology, and biochemical characteristics (Harrigan & MacCance, 1976; Kimaryo *et al.*, 2000). These initial tests make it possible and it to classified the isolation of genus based on the characteristics and identification of tests are described by (Harrigan & MacCance, 1976; Holzapfel and Schillinger, 1992). Pure bacterial isolates were besides examined for Gram reaction, cell morphology, catalase production, motility, acid production from glucose & growth at using different temperatures 10° C, 15° C, 37° C, and 45° C according to these methods described by (Kebede *et al.*, 2007). Growth at the presence of NaCl (2, 3, 4, and 6.5) and pH different levels (4.5, 5.5, and 6.5). Cell morphology of gram-positive, rod-shaped, and cocci shaped, catalase-negative, non-motile, NH_3 from arginine, CO_2 from glucose, dextran production from sucrose. characteristic isolates are known to be bacteria with lactic acidity. The fermentation of Glucose, Sucrose, Arabinose, Mannose, Xylose, Galactose, Lactose, Raffinose, Esculin, and Sorbitol was tested on isolated lactic acid bacteria (Holt *et al.*, 1994). According to Bergey's Manual of Determinative Bacteriology, all LAB strains were described and classified to the genus level (Holt *et al.*, 1994).

RESULTS AND DISCUSSION

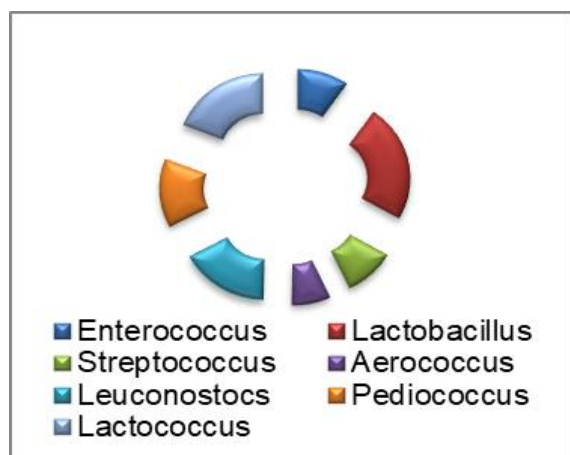
A total of Ninety isolates of lactic acid bacteria from thirty raw cow milk samples were collected from the City of Chidambaram surrounding area were classified and identified to the genus level. Isolated genus physiological, morphological, and biochemical characteristics are shown in (Table 1).

Table 1 Morphological, Physiological, and Biochemical Characterization of isolated genera of Lactic acid bacteria

Tests	Enterococcus	Lactobacillus	Streptococcus	Aerococcus	Leuconostocs	Pediococcus	Lactococcus
Cell Morphology	Round/cocci	Rods	Cocci/Chain	Cocci	Cocci/Ovoid	Round/cocci	Round/cocci
Gram staining	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-
Catalase activity	-	-	-	-	-	-	-
NH ₃ from arginine	-	+	-	-	-	-	-
CO ₂ from glucose	+	+	-	-	+	-	+
Dextrose Production	-	-	-	-	-	-	-
Acid Production from							
Glucose	-	+	+	+	+	-	+
Xylose	±	±	+	+	+	±	+
Melibiose	±	±	-	-	-	-	-
Sorbitol	-	+	-	-	±	±	+
Lactose	+	+	±	+	+	+	+
Sucrose	+	+	+	±	+	±	-
Raffinose	+	-	-	-	-	±	-
Galactose	+	±	+	+	-	-	-
Mannose	-	+	±	-	-	-	+
Esculin	-	±	-	+	-	-	+
Arabinose	+	-	-	±	+	+	-
Growth at Temperature (°C)							
10°C	-	-	-	-	-	-	±
15°C	+	+	-	-	-	-	+
37°C	+	+	+	+	-	+	+
45°C	±	±	-	±	±	±	±
Growth at (NaCl %)							
2%	+	-	+	+	+	±	+
3%	-	+	±	-	-	-	+
4%	+	±	-	-	+	+	+
6.5%	±	-	-	+	-	-	-
Growth at pH							
4.5	+	-	-	-	-	+	-
5.5	-	+	-	+	+	-	+
6.5	+	+	+	+	+	+	-

+ = Positive, - = Negative, ± = Varies between isolates.

All the isolates are Gram-positive and non-motile catalase. Microscopic observation was used to determine the cell morphology of all the isolates and the majority (N=66) were observed to be cocci, and the remainder (N=24) were rod-shaped. The isolates were classified into seven genera of lactic acid bacteria by analyzing the findings of physiological, morphological, and biochemical studies. This divided the isolates into *Lactobacillus* (29.32%), *Lactococcus* (23.68%), *Pediococcus* (17.09%), *Leuconostoc* (21.09%), *Streptococcus* (12.09%), *Enterococcus* (12.06%), and *Aerococcus* (9.09). The number of isolates grouped in the LAB genera identified is shown in (Figure 1).

**Figure 1** Different isolated genera of Lactic acid bacteria

LABs are coccus or rod-shaped bacteria with a catalase-negative, non-motile, hetero-fermentative, or homo-fermentative and low acid growth formers (Holzapfel et al., 2001). One of the microbes that dominate the fermented food is the lactic acid bacteria (LAB) (Guasch et al., 2005; Robert., 2008). Lactic acid bacteria (LAB) belong to a group of bacteria that are Gram-positive that produce lactic acid in the culture medium as their key fermentation tool and are widely recognized as healthy (Konings, 2000). Only the genus *Lactococcus* and *Lactobacillus* were able to produce carbon dioxide from the fermentation of glucose in the isolates. This has proven they are LAB heterofermentative. Only a few *Lactococcus* isolates in the genus grew at 10° C. All the isolates were able to expand in 2 percentage of NaCl except for any variation in the *Lactococcus* genus. By contrast, none of the isolates increased in NaCl of 6.5 %. The capacity of the isolates to expand at 4 percent NaCl and 45° C was distinct. All the isolates in the *Leuconostoc* genus, however, did not expand at 4 percent NaCl and 45° C. Some isolates of the *Lactococcus* genera have reported positive development at 10° C. All isolates showed positive results for the glucose and lactose fermentation in the carbohydrate fermentation pattern. However, the fermentation of the other carbohydrates does have a difference. Positive for fermentation of Raffinose was only *Pediococcus* and *Enterococcus* among the isolates. For Melibiose, the genus *Streptococcus*, *Leuconostoc*, *Aerococcus*, *Lactococcus*, *Pediococcus* were negative but, for *enterococcus* and *lactobacillus* genera, it was strain-dependent and variable. The 28 isolates of the *Lactococcus* genus do not digest sucrose. Except in other genera, the fermentation of sucrose varies which was studied by (Tserovska, 2004), also showed that lactic acid bacterial species extracted from goat's milk were in the shape of cocci and rod, either clustered and chained, characterized by gram-positive, catalase-negative, resistant to acid, microaerophilic, not producing spore, and producing lactic acid as a fermentation product. As LAB is located in nutrient-rich substrates such as milk, fermented beverages, food, vegetables, they can be isolated and characterized. It has been

shown that lactic acid produced by LAB not only preserves the total preservation of food and beverages but can also facilitate the development of healthy microbiota in the intestine, as well as reducing pathogens. The LAB species are also common as beneficial organisms in the food industry. One of these microorganisms' most important contributions is the increased shelf life of fermented products isolated from lactic acid bacteria is also possible from other substrates such as typical fermented beverages and foods, sourdough. The genus of lactic acid bacteria found in this research was generally comparable to other studies. Isolated genera of this sample were in the agreement with (Vuysta & Vancanneyt, 2007) who reported that *Streptococcus*, *Pediococcus*, *Lactobacillus*, and *Leuconostoc* had been isolated from borde and Shamita. Additionally (Hirayama & Rafter, 1999) isolated *L. fermentum*, *Leuconostoc* spp, *Aerococcus* spp, *Pediococcus* spp, and *Enterococcus* spp were from the sourdough. Furthermore (Sawsan et al., 2010) found the genera *Lactococcus*, *Lactobacillus*, and *Pediococcus* from cow raw milk, cheese, and rob in Sudan. In the current study, *Lactobacillus* (29.32%) was the dominant genera isolated from cow raw milk. As a dominant isolate, the genera *Lactococcus*, *Leuconostoc*, and *Lactobacillus* included pyogenic *Streptococci*, *Aerococcus*, and *Enterococci* also identified from traditional butter from Dhan (Guessas et al., 2012). LAB produce lactic acid as the principal end product of the fermentation of carbohydrates. Lactic acid bacterial species are very important as beneficial organisms for use as a starter culture in the food industry. One of these microorganisms' most important contributions is the extend shelf-life of the fermented products. The genera *Pediococcus*, *Lactobacillus*, *Lactococcus* belong to the lactic acid bacteria, and the bacterial strains of this genera are often used to produce and preserve many foods or as probiotics for the humans and animals on a large scale (Holzapfel, 1998; Osmanagaoglu, 2010). The current study has had considerable success in the isolation of lactic acid bacteria from cow raw milk samples. The result showed that cow raw milk is a good source of bacteria for lactic acid.

CONCLUSION

The research was concluded the isolation and identification of naturally occurring lactic acid bacteria from cow raw milk samples. This is a total of ninety lactic acid bacteria belonging to the genus *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, and *Aerococcus* were identified from thirty cow raw milk samples. Results from this study have shown that there is a diversity of lactic acid bacteria in cow raw milk. Lactic acid bacteria is a presence in milk and milk products enhances the nutrient bioavailability and acts as a preservative. This study from, we can conclude from this research that cow raw milk is a good healthy source of lactic acid bacteria. Also, Raw cow milk has been shown to contain both heterofermentative and homofermentative bacteria of the lactic acid. In future work, this local raw milk may serve as a source of beneficial lactic acid bacteria. Further studies on characterization, species-level identification, and their probiotic potential will be done.

Acknowledgments: I am a non-stipendiary research scholar. I wish to express sincere thanks to the authority of Annamalai University for providing Lab facilities.

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