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Dominating Species of Lactobacilli and Leuconostocs Present Among The Lactic Acid Bacteria of Milk of Different Cattles

G. P. Singh and Rakesh Roshan Sharma*
Department of Botany,
University of Rajasthan,
Jaipur (Raj.); India.

Abstract : The presence of dominating *Lactobacilli* and *Leuconostoc* spp. in the different cattle milks was studied. Total viable counting and total coliform counting were also checked for all the samples. In samples of cow, buffalo, camel and goat's milk, total 6 *Lactobacilli* spp. and 5 spp. of *Leuconostoc* were identified on the basis of their biochemical prosperities. Highest count of total bacterial population was found in the cow's milk and lowest in the goat's milk. Buffalo's milk was found with a significant number of coliforms. Lactic acid bacteria, genus Lactobacilli and Leuconostocs were obsereved in all the milk samples at a little difference in number.

Key words : Biochemical properties, Cattle Milk, Coliforms, Lactobacilli, Leuconostocs.

Interest in microorganisms as a component of biological diversity has been renewed in recent years (Alsopp *et al*., 1995). The interest in microorganisms occurring in food is primarily due to the biotechnological potential of new bacterial species and strains (Leisner *et al*.,1999).

In the history, milk played a major role as nutritional source and since 1900's the start of golden era of industrial microbiology. It was also economically significant because larger quantity of milk was being processed daily in factories for the fermented food products.

Lactic Acid Bacteria (LAB) widely distributed in the nature and occurring naturally as indigenous microflora in raw milk that play an important role in many food and feed fermentations. Lactic acid bacteria are a group of Gram positive, non sporing, non respiring cocci or rods which produce lactic acid as the major end product during the fermentation of carbohydrates and are used as starter culture (Henneberg, 1904). In this group included representatives of the genus *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostocs*. The lactic acid fermentation, which these bacteria carry out, has long been known and applied by humans for making different foodstuffs. For many centuries LAB have served to provide an effective form of natural preservation. In addition , they strongly determine the flavor , texture and , frequently , the nutritional value of food and feed products.

Staphylococcus aureus is Gram positive, food borne pathogen capable of growing in foods at refrigeration temperature. It grows at temperatures between 5 and 12°C (Palumbo, 1986). *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of pyrogenic exotoxins into the blood stream. These conditions have initiated a search for naturally produced biopreservatives. One area that has generated much interest is the use of antimicrobial metabolites from lactic acid bacteria used in food fermentation. Some of the metabolites of these bacteria have an antimicrobial effect against many food spoilage

*** Corresponding author :** Rakesh Roshan Sharma, Department of Botany, University of Rajasthan, Jaipur (Raj.); India; E-mail: *daggur2s@yahoo.co.uk*

and pathogenic bacteria, include lactic acid, diacetyls, hydrogen peroxide, and proteinaceous substances bacteriocins (Barefoot and Klaenhammer 1983; Daeschel, 1989).Bacteriocin producing *Lactobacillus casei* previously identified and isolated from infant stool sample (Joshi and Chaudhary, 2003).

The aim of this work was the isolation and taxonomic determination of a large number of lactic acid bacteria from cattle's raw milk in order to constitute and original collection of LAB strains and to use them as a source of biopreservatives.

Materials and Methods

The milk samples were collected during the lactation process in sterile screw cap tubes and processed within 3 hours. The milk samples were shaken before diluting. The samples were diluted to $1{:}10^3$, $1{:}10^4$, $1{:}10^5$ by using sterilized phosphate buffered water. The diluted samples were shaken again by using sterile pipette each time and transferred a measured quantity (0.l ml) of the sample into each sterilized petriplate. Four standard count media Nutrient Agar, de-Man Rogosa Agar, Sodium Azide Agar , Eosine Methylene Blue Agar Media plates were prepared for each sample in replicates.
These media were used for enumeration of total bacteria, *Lactobacilli, Leuconostocs* and coliforms, respectively.

Thoroughly mixed the samples with media and the medium were allowed to solidify. After solidification the plates were incubated at 37°C for 24 hours. After incubation the colonies were counted and the results were recorded.

Few of the selected colonies were transferred into MRS and MMRS broth from MRS Agar and SA Agar, respectively. Cultivation was carried out with appropriate incubation temperature and time required for growth. It was 37° C for 24 hours for *Lactobacilli* and 25° C for 72 hours for *Leuconostocs*. Isolation of pure lactic acid bacteria cultures was completed by the streak plate method. Bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristic according to Bergeys Manual of Systematic Bacteriology (Williams, 1989).

Results and Discussion

Enumeration of microorganisms in different samples of cattle's milk by standard plate count method was accomplished, as presented in Table-1. The microbial colonies were counted in raw milk samples. The colonies in raw milk are expected a little higher than real microflora. This is due to contamination from the animal, especially the exterior of the udder and the adjacent area, bacteria found in manure, soil and water may enter (Garbutt, 1997).

From the tested samples forty lactic acid bacterial cultures were isolated to draw conclusion about the resident lactobacilli and *Leuconostoc species* of the milk of particular

Table 1 : Total Viable Counting on Various Agars (Average Colony Forming Units Of Replicates)

Sample	Media			
	NA	MRS	SA	EMB
Cow milk	8.8×10^5	1.2×10^5	0.8×10^5	0.2×10^5
Buffalo milk	7.0×10^5	1.4×10^5	0.9×10^5	1.5×10^5
Camel milk	7.5×10^5	1.6×10^5	1.3×10^5	0.3×10^5
Goat milk	5.0×10^5	1.0×10^5	0.7×10^5	0.2×10^5

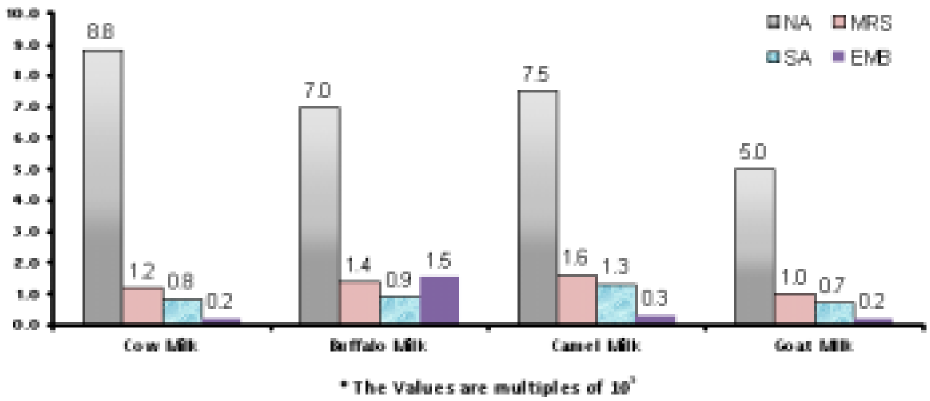

Fig. 1 : Total Bacterial Counting Results on Different Agars

cattles. *Lactobacilli* and *Leuconostocs* both were found higher in number in camel milk as compared to cow, buffalo and goat milk. However number of total bacteria was found higher in cow milk. Coliforms were found in significant number in buffalo milk (Figure: 1).

The study was conducted to conclude about the common or characteristic *Lactobacilli* and *Leuconostoc* species present in the particular milk. On the basis of biochemical test's identification results, it was concluded that both cow and buffalo milk's dominant *Lactobacilli spp*. is *Lb. brevis*. However *Lb. casei* and *Lb. fermentum* were also found in cow milk and *Lb. lactis* and *Lb. acidophilus* in buffalo milk. *Lb. acidophilus* was found dominating in camel milk with a few *Lb. casei* and *Lb. fermentum. Lb. delbrueckii* was dominating *Lactobacilli spp*. of goat milk with a less number of *Lb. lactis* (Table-3).

In case of *Leuconostoc spp*., *Ln. dextranicum* was dominating the *Ln. lactis* and *Ln. paramesenteroids* in cow milk, *Ln. lactis* was dominating the *Ln. mesenteroids* and *Ln. cremoris* in buffalo milk, *Ln. cremoris* was dominating the *Ln. lactis* and *Ln. mesenteroids* in camel milk and *Ln. lactis* was dominating the *Ln. dextranicum* and *Ln. paramesenteroids* in goat milk (Table-4).

Compared to cow, buffalo and ewe milk fat, camel milk fat contains less short-chained fatty acids, but the same long chained fatty acids can be found (Dingra, 1934) . Gast (1969) claim that the value of camel milk is to be found in the high concentrations of volatile acids and, especially, linoleic acid and the polyunsaturated acids, which are essential for human nutrition, and this may be one of the factors for different species in different milk.

Data concerning the composition of milk vary greatly. This can be partly attributed to the inherited capabilities of the animals, but the stage of lactation, age, and the number of calvings also play a role. Of special significance to the quality of the produced milk are the feed and water quantity and quality.

Cow milk contains 3.5% to 5% fat. Milk also contains minerals like calcium, phosphorus which are in plenty and potassium, sodium, iodine in traces . It is an excellent source of Vitamin A, B, D, E and K. Carbohydrate acts as a sweetener and is a major source of energy. The specific gravity of camel milk is less than that of cow, sheep or buffalo milk (Shalash, 1979).

In the dairy products of cow and goat's milk including cheese and curds (leben and rayeb) the species composition of lactic acid

Table 2 : Group identification tests for Lactobacilli

Sl.	Isolates	Growth at		Gas from glucose	NHB form glucose	Catalase	Litmus Reaction			Remark
		15^0 C	45^0 C				A	R	C	
1.	CLb_1	+	-	-	-	-	+	+	+	Streptobacterium
2.	CLb_2	+	-	+	+	-	+	+	+	Betabacterium
3.	CLb_3	-	+	+	+	-	+	+	+	Betabacterium
4.	CLb_4	+	-	+	+	-	+	+	+	Betabacterium
5.	CLb_5	+	-	+	+	-	+	+	+	Betabacterium
6.	CLb_6	-	-	+	+	-	+	+	+	Betabacterium
7.	CLb_7	+	-	+	+	-	+	+	+	Betabacterium
8.	CLb_8	+	-	+	+	-	+	+	+	Betabacterium
9.	CLb_9	-	-	+	+	-	+	+	+	Betabacterium
10.	CLb_{10}	+	-	+	+	-	+	+	+	Betabacterium
11.	BLb_1	+	-	+	+	-	+	+	+	Betabacterium
12.	BLb_2	+	+	-	-	-	+	+	+	Thermobacterium
13.	BLb_3	-	+	-	S+	-	+	+	+	Thermobacterium
14.	BLb_4	-	+	+	+	-	+	+	+	Betabacterium
15.	BLb_5	+	+	-	-	-	+	+	+	Thermobacterium
16.	BLb_6	-	+	-	S+	-	+	+	+	Thermobacterium
17.	BLb_7	-	+	+	+	-	+	+	+	Betabacterium
18.	BLb_8	-	+	-	-	-	+	+	+	Thermobacterium
19.	BLb_9	+	+	-	-	-	+	+	+	Thermobacterium
20.	BLb_{10}	-	+	+	+	-	+	+	+	Betabacterium
21.	$CmLb_1$	+	-	+	-	-	+	+	+	Streptobacterium
22.	$CmLb_2$	-	+	+	+	-	+	+	+	Betabacterium
23.	$CmLb_3$	+	+	-	-	-	+	+	+	Thermobacterium
24.	$CmLb_4$	-	+	+	+	-	+	+	+	Betabacterium
25.	$CmLb_5$	+	+	-	-	-	+	+	+	Thermobacterium
26.	$CmLb_6$	+	+	-	-	-	+	+	+	Thermobacterium
27.	$CmLb_7$	-	+	+	+	-	+	+	+	Betabacterium
28.	$CmLb_8$	+	+	-	-	-	+	+	+	Thermobacterium
29.	$CmLb_9$	+	+	-	-	-	+	+	+	Thermobacterium
30.	$CmLb_{10}$	+	-	-	-	-	+	+	+	Streptobacterium
31.	GLb_1	-	+	+	+	-	+	+	+	Betabacterium
32.	GLb_2	-	+	-	S+	-	+	+	+	Thermobacterium
33.	GLb_3	-	+	-	S+	-	+	+	+	Thermobacterium
34.	GLb_4	-	+	-	S+	-	+	+	+	Thermobacterium
35.	GLb_5	+	+	-	-	-	+	+	+	Thermobacterium
36.	GLb_6	-	+	-	S+	-	+	+	+	Thermobacterium
37.	GLb_7	-	+	-	S+	-	+	+	+	Thermobacterium
38.	GLb_8	-	+	-	S+	-	+	+	+	Thermobacterium

CLb - Cow *Lactobacillus*, BLb - Buffalo *Lactobacillus*, CmLb - Camel *Lactobacillus*, GLb - Goat *Lactobacillus*

Table 3 : Species Identification Tests For Lactobacilli-

SL	Isolates	VP	Arab-inose	Celli-biose	Lacto-se	Man-nitol	Meli-biose	Salic-in	Sorbi-tol	Surc-ose	Raffi-nose	Treh-alose	Remark
1	CLb$_1$	+	-	+	-	+	-	+	+	+	-	+	*Lb. Casei*
2	CLb$_2$	-	-	-	S+	-	-	-	-	S+	S+	-	*Lb. brevis*
3	CLb$_3$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
4	CLb$_4$	-	-	-	S+	-	+	-	-	S+	S+	-	*Lb. brevis*
5	CLb$_5$	-	-	-	S+	-	-	-	-	S+	S+	-	*Lb. brevis*
6	CLb$_6$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
7	CLb$_7$	-	-	-	S+	-	-	-	-	S+	S+	-	*Lb. brevis*
8	CLb$_8$	-	-	-	S+	-	+	-	-	S+	S+	-	*Lb. brevis*
9	CLb$_9$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
10	CLb$_{10}$	-	-	-	S+	-	-	-	-	S+	S+	-	*Lb. brevis*
11	BLb$_1$	S+	-	-	S+	-	+	-	-	S+	S+	-	*Lb. brevis*
12	BLb$_2$	+	-	S+	S+	-	-	+	+	+	-	+	*Lb. lactis*
13	BLb$_3$	+	-	S+	+	-	-	+	+	+	-	+	*Lb. lactis*
14	BLb$_4$	-	-	-	S+	-	+	-	-	S+	S+	S+	*Lb. brevis*
15	BLb$_5$	+	-	S+	S+	-	-	+	+	+	-	+	*Lb. lactis*
16	BLb$_6$	+	-	S+	+	-	-	+	+	+	-	+	*Lb. lactis*
17	BLb$_7$	-	-	-	S+	-	+	-	-	S+	S+	S+	*Lb. brevis*
18	BLb$_8$	+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
19	BLb$_9$	+	-	S+	S+	-	-	+	+	+	-	+	*Lb. lactis*
20	BLb$_{10}$	-	-	-	S+	-	+	-	-	S+	S+	S+	*Lb. brevis*
21	CmLb$_1$	+	-	+	S+	+	-	+	+	+	-	+	*Lb. casei*
22	CmLb$_2$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
23	CmLb$_3$	S+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
24	CmLb$_4$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
25	CmLb$_5$	S+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
26	CmLb$_6$	S+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
27	CmLb$_7$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
28	CmLb$_8$	S+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
29	CmLb$_9$	S+	-	+	+	-	S+	+	+	+	S+	S+	*Lb. acidophilus*
30	CmLb$_{10}$	+	-	+	-	+	-	+	+	+	_	+	*Lb. Casei*
31	GLb$_1$	+	-	S+	+	-	+	-	-	+	+	S+	*Lb. fermentum*
32	GLb$_2$	+	-	S+	-	-	-	-	+	+	-	S+	*Lb. delbrueckii*
33	GLb$_3$	+	-	S+	+	-	-	+	+	+	-	+	*Lb. lactis*
34	GLb$_4$	+	-	S+	-	-	-	-	-	+	-	S+	*Lb. delbrueckii*
35	GLb$_5$	+	-	S+	S+	-	-	+	+	+	-	+	*Lb. lactis*
36	GLb$_6$	+	-	S+	+	-	-	+	+	+	-	+	*Lb. lactis*
37	GLb$_7$	+	-	S+	-	-	-	-	-	+	-	S+	*Lb. delbrueckii*
38	GLb$_8$	+	-	S+	+	-	-	+	+	+	-	+	*Lb. lactis*
39	GLb$_9$	+	-	S+	-	-	-	-	+	+	-	S+	*Lb. delbrueckii*
40	GLb$_{10}$	+	-	S+	-	-	-	-	-	+	-	S+	*Lb. delbrueckii*

CLb - Cow *Lactobacillus,* BLb - Buffalo *Lactobacillus,* CmLb - Camel *Lactobacillus,* GLb - Goat *Lactobacillus*

Table 4 : Species identification tests for Leuconostocs-

Sl.	Isolate	Growth at 37°C	Gas from glucose	NH_3 Prodn	Catalase test	Litmus A	Litmus R	Litmus C	Growth at 4.5 pH	Heat resistance	CU Test	Dex. Prodn	CO_2 prodn	VP Test	Arb.	Suc.	Trh.	Spp.
1	CLn1	+	-	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
2	CLn2	S+	-	-	-	+	+	+	-	-	S+	-	-	+	-	+	+	*Ln. paramesenteroides*
3	CLn3	+	S+	-	-	+	+	+	-	+	S+	-	+	-	-	+	-	*Ln. lactis*
4	CLn4	+	S+	-	-	+	+	+	-	-	S+	+	-	-	-	+	+	*Ln. dextranicum*
5	CLn5	+	-	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
6	CLn6	S+	-	-	-	+	+	+	-	-	S+	-	-	+	-	+	+	*Ln. paramesenteroides*
7	CLn7	+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
8	CLn8	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
9	CLn9	S+	-	-	-	+	+	+	-	-	S+	-	-	+	-	+	+	*Ln. paramesenteroides*
10	CLn10	+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
11	BLn1	+	-	-	-	+	+	-	-	+	-	-	-	S+	-	+	-	*Ln. lactis*
12	BLn2	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
13	BLn3	S+	+	-	-	+	+	+	-	-	+	+	S+	-	-	+	+	*Ln. mesenteroides*
14	BLn4	S+	+	-	-	+	+	+	-	-	+	+	S+	-	-	+	+	*Ln. mesenteroids*
15	BLn5	+	S+	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
16	BLn6	+	-	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
17	BLn7	+	S+	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
18	BLn8	S+	+	-	-	+	+	+	-	-	+	+	S+	-	-	+	+	*Ln. mesenteroids*
19	BLn9	+	S+	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*
20	BLn10	S+	+	-	-	+	+	+	-	-	+	+	S+	-	-	+	+	*Ln. mesenteroides*
21	CmLn1	S+	+	-	-	+	+	+	-	-	+	+	++	-	-	+	+	*Ln. mesenteroides*
22	CmLn2	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
23	CmLn3	+	S+	-	-	+	+	+	-	+	-	-	S+	-	-	+	-	*Ln. lactis*
24	CmLn4	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
25	CmLn5	S+	+	-	-	+	+	+	-	-	+	+	++	-	-	+	+	*Ln. mesenteroides*
26	CmLn6	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
27	CmLn7	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
28	CmLn8	+	S+	-	-	+	+	+	-	+	-	-	S+	-	-	+	-	*Ln. lactis*
29	CmLn9	-	+	-	-	+	+	+	-	S+	+	-	+	-	-	-	S+	*Ln. cremoris*
30	CmLn10	+	S+	-	-	+	+	+	-	+	-	-	S+	-	-	+	-	*Ln. lactis*
31	GLn1	+	S+	-	-	+	+	+	-	-	S+	+	-	-	-	+	+	*Ln. dextranicum*
32	GLn2	+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
33	GLn3	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
34	GLn4	+	S+	-	-	+	+	+	-	+	S+	-	+	-	-	+	-	*Ln. lactis*
35	GLn5	+	S+	-	-	+	+	+	-	-	S+	-	-	+	-	+	+	*Ln. paramesenteroides*
36	GLn6	+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
37	GLn7	+	-	-	-	+	+	+	-	-	+	-	S+	S+	-	+	-	*Ln. lactis*
38	GLn8	+	S+	-	-	+	+	+	-	+	S+	-	+	-	-	+	-	*Ln. lactis*
39	GLn9	+	-	-	-	+	+	+	-	-	-	+	-	-	-	+	+	*Ln. dextranicum*
40	GLn10	+	-	-	-	+	+	+	-	+	+	-	S+	S+	-	+	-	*Ln. lactis*

CLb - Cow *Lactobacillus*, BLb - Buffalo *Lactobacillus*, CmLb - Camel *Lactobacillus*, GLb - Goat *Lactobacillus* bacteria is more varing and inconsistent when compared with those of the trade products. In biotechnological aspect, the "wild" strains of the LABs are prospective bacteriocin producers (Niku-paavola *et al*., 1999; Park *et al* ., 2003) and probiotic (Rinkinen *et al*.,2003).

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