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ORIGINAL RESEARCH ARTICLE

Microbiological and Biochemical Characteristics of Tradition Dairy Product: Identification of Dominant *Lactobacillus*

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ABSTRACT

A total of 27 strains were isolated from 5 Traditional Dairy Products samples that were collected in Pondicherry. The Lactic acid bacteria (LAB) dominated the Microbial Population of Dairy Samples and were identified on basis of their morphological, physiological and biochemical characteristics. Among the isolates, the *Lactobacillus* LBC216 was predominant. The distribution of the isolates by was as follows; *Lactobacillus* spp LBC 216 (10%), LBL 217 (4%), LBB 218 (2%), LHB 219 (2%), LBF 220 (2%). The five representative *Lactobacillus* spp were identified to species level in carbohydrate pattern according to Bergey's Manual. The identified strains were then evaluated for some technological properties. One strain *Lactobacillus* LBC 216 only had antagonistic properties against *Staphylococcus aureus* and *Bacillus cereus*.

Key words: Dairy products, Lactic acid bacteria, *Lactobacillus* Species, Antagonistic activity, food pathogens.

INTRODUCTION

Milk products made from locally produced raw milk and it was very important part of the daily diet; the nature of these products is different from one region to another depending on the local indigenous micro

Flora, which in turn reflects the climatic conditions of the area. These Dairy products have one feature in common fermentation by lactic acid bacteria (LAB) is an integral part of their manufacture.

Curd, Butter, Milkpeda, Cheese and Ghee are Traditional Dairy products in rural areas, these products are traditionally made from raw cow milk. These samples are left to room temperature it contains a mixed culture of LAB and other fermentative organisms. Lactic acid bacteria (LAB) commonly used as a natural food preservative to improve the food safety and stability. Among to LAB, Lactobacilli are the most important group and are gaining increasing attention in food fermentation industry because of their potential biotechnological interest [1,2].

Nowadays, consumers demand for processed dairy products besides consistency in overall quality. Therefore, the dairy industry is kept on increasing and important for expanding the diversity of its product range. Accordingly, LAB recoverable from raw milk or Dairy Products [3,4]. These products are produced some bacteriocin antagonistic towards closely related species and pathogen [5].

Therefore, the isolation and characterization of new strain from the Traditional Dairy Products is necessary in order to bring novel strain to the industry. Phenotypic methods relaying on physiological and biochemical criteria have been widely used for Lactobacillus Spp, identification, the use of phenotypic mean of identification of LAB from different sources has been reported [6].

The objective of this study was to characterize and identify dominant Lactobacillus that occur naturally in Traditional Dairy products by using both physiological and biochemical methods, antagonistic activity and to determine their technological properties.

MATERIALS AND METHODS

Collection of Samples:

Five samples (Curd, cheese, Butter, Milk Peda and Ghee) made by Traditional Method were collected aseptically from local procedures from Pondicherry, samples were brought to the laboratory and stored in under refrigeration at 4º C unit used.

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Microbial Population Counting

Lactic acid bacterial count was estimated using Tomato Juice agar medium as described by oxoid manual. Coliform counts was counted using EMI agar. mould and yeasts count were determined using potato dextrose agar.

Isolation of lactic acid bacteria:

10 ml of each Dairy product were aseptically added into 90 ml of sterile 0.9% Nacl Solution and mixed thoroughly serial dilutions (10^{-1} to10^{-7}) performed and 1ml aliquots of appropriate dilution were directly inoculated in triplicate on the following media:

a) MRS agar incubated aerobically for 48 h 37ºC for isolation of *Lactobacilli* and *Lactococcus* [8].

b) M 17 agar, incubated aerobically for 48 h at 37ºC for isolation of *Enterococci* and *Streptococcus*.

c) MRS agar containing 10 ugml^{-1} vancomycin incubated anaerobically for 72 h at 25ºC for isolation of *Leuconostoc* [9].

After incubation, all colonies from plates representing 5–10 colonies were further purified by successive streaking on the corresponding agar. Five purified isolates were obtained from the above mentioned samples. These isolates were preserved in 15% (v/v) glycerol and stored at -20ºC until further tests.

Screening and identification of *Lactobacillus* Species:

All isolates (Five) were microscopically examined for gram stain reaction, cell morphology and cellular arrangement [10]. Catalase activity and production of acid from glucose [11] were also determined to identify the isolates at the genus level only Gram positive and catalase negative isolates were identified at species level.

Antagonistic activity:

Lactobacillus Spp isolates were screened for antibacterial activity by the agar well diffusion method. An overnight culture of pathogens grown in TSB Medium at 30ºC was diluted to a turbidity equivalent to that of a 3.0 McFarland Standard with a sterilized 0.85% Nacl Solution. A lawn of an indicator strain was made by spreading the cell suspension over the surface of BHI plates with a cotton Swab, the plates were allowed to dry and a sterile cork borer of diameter 5 mm was used to cut uniform wells in the agar

plates. Each well was filled with 50 microlitre of filter sterilized supernatant obtained from culture grown in MRS Medium. All the assays were carried out in triplicate. After incubation at 37ºC for 24 h, the diameter (mm) of the inhibition zone around the well was measured.

RESULTS AND DISCUSSION

In this study of *Lactobacillus* Spp isolated from different Dairy products such as curd, cheese, butter, milk peda and ghee were characterized. The mean log counts (cfug^{-1}) of the dominant microbial groups of dairy products collected from Pondicherry are summarized in (**Table 1**). Counts of coliform, mould and yeasts were high in all samples and ranged from 1.0 ± 0.01 to 3.8 ± 0.05. All these populations rose from around 10^2 cfug^{-1} to 10^4 cfug^{-1} . These results can be explained by the fact that the methods of production of the various traditional foods are usually primitive and the major risk enhancing factors are the use of contaminated raw materials, lacks of pasteurization and inadequate fermentation and storage conditions [12].Despite the large number of different bacterial species of the lactic acid bacteria (LAB) group dominated the microbiota, in all samples to reach a final population of around 10^6 to 10^7 cfug^{-1}.LAB was the predominant microbial group in Dairy products, which is important because of the key role it plays in fermentation processes and its production of antagonistic properties, as well as its potential use as a co-starter culture and biopreservative of foods. For these reasons, LAB strains were isolated and identified.

Twenty seven Lactic acid bacteria isolated from Dairy samples were identified phenotypically. Among Five Lactobacillus species were grouped on the basis of gram stain reaction, cell shape, cellular arrangement, and production of acid from glucose, gas from glucose and catalase activity. Based on Taxonomic characteristics (**Table 3**), the majority of isolates were Gram-positive and catalase-negative. Summarizes the results above described and distribution of isolates from the different Media as follows (**Table 2**): 11 isolates on M17 15 isolates on MRS agar and 1 isolates on MRS+V agar the selectivity of MRS medium was 90% for Lactobacilli and it was < 10% for other genera, similar results were reported in [9] who found that the estimated selectivity was 67.1, 59.3 and 93.6% for M_{17}, MRS and MRS +V respectively. (**Fig 1**) illustrates the distribution at genus level of 27 LAB identified from Traditional Dairy products. The isolates were divided into five genera: *Lactobacilli* (20%) *Streptococcus* (15%). *Lactococcus* (8%), *Enterococcus* (5%) and *Leuconostoc* (2%). Similar results were

reported by [13] and [14] who identified six general from Traditional fermented milk: *Leuconostoc, Lactococcus, Lactobacillus, Enterococcus, Streptococcus* and *Pediococcus*.

The dominance of *Lactobacillus* among the isolated strains is consistent with the finding of[15] *Lactobacillus* is able to survive in highly acidic environment of pH = 4 to 6.5 or over lower, and due to these properties *Lactobacillus* is responsible for final stages of fermentation in the products. This further showed that low pH conditions favor the growth of Lactobacillus [16].

Identification of *Lactobacillus* species were identified on basis of their morphological characteristics, gas and acid production from glucose (**Table 4**). Based on phenotypic characteristics ten strains were satisfactorily identified. (To compare *Lactobacillus casei* MTCC 1432) of which Five were identified using a sugar utilization profile. (Table 4) these strain was tentatively identified as Lactobacillus *casei*, totally *Lactobacillus* strains (20.0%) were classified into 10 isolates. LBC216 was the predominant species (10%), LBL217 (4%), LBB218 (2%), LBH219 (2%), LBF220 (2%), similar results were obtained by[14] and[17], who found that 33.3% and 26.3% of isolates from Sudanese Sour Milk and Traditional Rayeb Milk was general *Lactobacillus.* The predominance of *Lactobacillus* genera due to these types of bacteria are commonly associated with the warm climatic condition of productive regions[18], while *Lactobacillus* strains (LBB218, LBH 219and LBF220) were found at low level because they are fermentative culture, complex nutritional requirements and show a weak competitiveness during milk Fermentation[19].

The culture of *Lactobacillus* species; that included 5 isolates *Lactobacillus casei* 216, LBL217, LBB218, LBH219 and LBF220 capable of assimilation. On occasion, differences are also due to the presence on absence of nutrient transport systems [20]. The extracts of five isolated from Traditional Dairy products were tested by well diffusion method to know if the antagonistic properties produced. From the results (**Table 5**). It could be noticed that none of LBL217, LBB, 218 LBH219 and LBF220 isolates showed antibacterial activity against indicator strain of *S. aureus* and *B. cereus.* Among the LBC216 (*Lactobacillus casei*) strain showed, Antagonistic activity against *S. aureus* and *B cereus.* This suggests that antimicrobial properties of such strains can reduce the number of other undesired microorganisms in milk essential role in the preservation of product for human consumption [21]

Table 1: Microbial Viable Count (cfug^{-1}) found in Dairy sample obtained from Pondicherry.

Microbial Count	Dairy Products				
	Curd	Cheese	Butter	Milkpeda	Ghee
LAB	4.5±0.05^{a}	3.9±0.10^{a}	2.5±0.05^{b}	3.0±0.15^{a}	3.5±0.05^{a}
Coliform	3.8±0.05^{b}	3.1±0.15^{b}	2.6±0.09^{b}	2.8±0.02^{b}	3.2±0.28^{b}
Fungi *	1.8±0.02^{c}	1.1±0.14^{c}	1.0±0.01^{c}	1.2±0.16^{c}	1.1±0.38^{c}

* = Yeast, Mould

Values are mean ±SD of three replications. Each experiment is repeated three times and similar results are obtained each time. Means followed by different letters are differed significantly according to lease significant difference test (P>0.05)

Table 2: Grouping of representative strains of LAB isolated from Traditional Dairy products using different Media

Genus	Culture Medium *			
	M17	MRS	MRS+V	Total
Streptococci	5	2	+	7
Lactobacilli	-	10	-	10
Lactococci	2	3	-	5
Enterococci	4	-	-	4
Leuconostoc	-	-	1	1
Total	11	15	1	27

*Culture Media utilized for counting of the different lactic acid bacteria group :

M17 for *Streptococci,* MRS for *Lactobacilli*,*Lactococcus,* MRS + plus vancomycin (microgram ml) for *Leuconostoc.*

Table 3: Taxonomic properties of *Lactobacillus* Spp isolated from Traditional Dairy products.

Characteristics	Groups					
	LBC216	LBL217	LBB218	LBH219	LBF220	*Lactobacillus casei* MTCC 1432
No. isolates	5	2	1	1	1	1
Gram Stain	+	+	+	+	+	+
Shape	rod	rod	rod	rod	rod	rod
Catalase	-	-	-	-	-	-
Gas from glucose	0/5^{a}	0/2	0/1	0/1	0/1	-

Growth at 10ºC	NT	NT	NT	NT	NT	-
15 ºC	0/5	0/2	1/1	0/1	0/1	+
20 ºC	0/5	2/2	1/1	1/1	1/1	+
30 ºC	5/5	2/2	1/1	1/1	1/1	+
40 ºC	5/5	0/2	1/1	1/1	NT	+
pH 3.5	0/5	2/2	1/1	0/1	0/1	-
pH 4.5	0/5	2/2	0/1	1/1	0/1	-
PH 5.5	5/5	1/2	1/1	0/1	1/1	+
pH 7.0	5/5	2/2	1/1	0/1	1/1	+
pH 8.5	0/5	0/2	1/1	1/1	1/1	-
Spores formation	-	-	-	-	-	-
H2 S Formation	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-
urease Co_2 from glucose	-	-	-	-	-	-

[a] Number of positive strains / total number of strains

NT : Not tested; + : Positive; - : Negative

Table 4: Isolates based on Characterization of Carbohydrate utilization from different *Lactobacillus* Spp

S.No	Sugar	LBC216	LBL217	LBB218	LBH219	LBF220	*L.casei*MTCC1432
1.	Maltose	+	+	-	*	+	+
2.	Mannose	+	+	-	+	+	+
3.	Mannitol	-	*	-	-	-	-
4.	Fructose	+	+	+	-	+	+
5.	Galactose	+	+	-	+	+	+
6.	Lactose	+	+	+	+	+	+
7.	Sucrose	-	+	-	-	+	-
8.	Xylose	-	+	-	-	-	-
9.	Sorbitol	-	+	-	-	-	-
10.	Glucose	+	+	+	+	+	+

+ = Good growth; - = No growth; * = Poor growth;

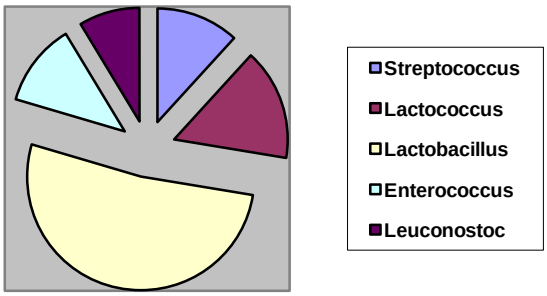
Table 5: Inhibitory spectrum of Lactobacillus Spp, isolated from Traditional Dairy Products.

Indicator Stains	Inhibition Zones (mm) from culture				
	LBC216	LBL217	LBB218	LBH219	LBF220
Lactobacillus acidophilus	-	*	-	+	-
Streptococcus Sp.,	-	-	+	-	-
Bacillus cereus	++	+	-	-	-
Bacillus substilis	*	-	-	+	+
Staphylococcus aureus	+++	-	-	-	-
Pseudomonas aeruginosa	-	-	*	-	-
Salmonella typhi	*	-	-	-	-
Escherichia coli	*	+	-	-	-
Shigella dysenteriae	-	-	+	-	-

All indicator strains were cultured in BHI Medium, Inhibition Zone of at least 5mm in diameter.

- = No inhibition activity; * = Weak inhibition zone; +, ++ = Good inhibition zone; +++ = Very good inhibition zone

Fig1. Distribution of LAB at genus level isolated from Traditional Dairy products.



CONCLUSION

This study on traditional dairy products showed that LAB; *Lactobacillus casei* LBC216 are the dominant Antagonistic activity, which have a significant effect on the overall quality of Dairy products. Some of the isolated and identified Lactobacillus Spp showed outstanding performances that were similar to commercially available cultures. The further study will be focus on the genotypic characterization of these isolates.

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