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Lipolytic *Lactobacillus* Species from Camel Milk

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Authors' contributions

This work was carried out in collaboration between both authors. Author MR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KS managed the analyses of the study and reviewed the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Over the past few years, lactic acid bacteria (LAB) have received extensive consideration as probiotics. Probiotics concepts have gained so much of popularity and extend from traditional dairy products to a profitable market of probiotic health supplements and functional foods. Probiotics are known for their health benefits as well as their human friendly nature. Probiotic lactic acid bacteria with lipolytic activity can be helpful for people's suffering from high serum lipid level. Consumption of such products contained lipolytic probiotic lactic acid bacteria can help to reduce the occurrence of consequences associated with high serum level. In the present work, two bacterial isolates were purified from camel milk. Sequencing of 16S rDNA/D1/D2 domain of LSU rDNA or ITS region and BLAST analysis lipolytic bacterial isolates were identified as *Lactobacillus plantarum and Bacillus,* Innovation of this work is use of camel milk sample for isolation of lipid degrading probiotic *Lactobacillus* bacteria which is easily available in Rajasthan region. *Lactobacillus spp* purified from screened sample showed significant lipase activity hence probiotic formulation developed from the same can be helpful for those people suffering from complications associated with high lipid level.

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1. INTRODUCTION

Lactic acid bacteria are generally recognized as safe (GRAS) group of bacteria. They are catalase negative, cocci or rod shaped bacteria, give positive Gram staining reaction. Food and dairy samples are most likely to contain lactic acid bacteria. Foods can directly or after enriching with the probiotic isolates, can be used as a probiotic supplementary food. Probiotics are live microorganisms that have health benefits when consumed. Several probiotic items are available in market. These products are very helpful in building up immunity as well as digestive health, reduce depression and promote heart health.

Present work deals with the isolation of lipolytic isolates from fermented food and dairy samples. *Lactobacillus* from these samples regarded as safe. *Lactobacillus* isolates are usually used to develop probiotic formulation. Probiotic microorganisms can be screened from non-intestinal sources, such as fermented food [1], fruit juices [2], grains [3], honey-comb [4] and soil [5,6]. LAB primarily *Lactobacillus plantarum* has been found in many types of fruit juices from both solid and citrus fruits whereas *Leuconostoc mesenteroides* is rarely found in these fruits but is the species that is most commonly found in tomatoes [2]. Literature suggested the presence of lipolytic lactic acid bacteria from camel milk and other dairy sources [7,8]. The alternative growth medium that is used to cultivate lactic acid bacteria can also be used to select probiotics from sources such as pineapple wastes [9] and tomato juice [10]. LAB can be found in food products stored at a low temperature (4°C) such as vacuum-packaged beef and some beneficial isolates can be screened in a similar way as bacteria that produce bacteriocin-like substance [11].

Camel milk is easily available in Rajasthan region, known for therapeutic and nutritional properties. Presence of probiotic *Lactobacillus* in the same can further increase its importance. Fortified camel milk enriched with *Lactobacillus* species can be used for the heart patient or reduction of implications associated with the same. Determination of probiotic properties of *Lactobacillus plantarum* identified in camel milk and further quality assurance will pave the way to develop formulation.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Molecular Typing of Lipolytic Lactic Acid Bacteria

2.1.1 Strains purified from fermented food and camel milk

Fresh camel milk collected from Dhani located in Udaipur (Rajasthan) in pre-sterlized ampule. Half of the portion subjected to serial dilution and subsequently on MRS agar to isolate *Lactobacillus* bacteria while left over portion kept at room temperature for fermentation.

Two bacterial isolates were isolated and purified from camel milk samples.

Commercialized products positively contained probiotic strains like yakult (Japan) were also subjected to isolation process. Purified bacteria isolates were identified by direct microscopic examination, cultural characteristics and biochemical tests. In all the cases, phenotypic identification was done following the Bergey's manual of determinative biology [12]. Purified bacterial colonies were observed for microscopic morphology (Gram-positive, Gram-negative, shape and arrangement etc.).

2.2 Molecular Identification of Bacterial Isolate

2.2.1 DNA extraction

For DNA extraction, single colonies were resuspended in 50 µL of sterile deionized water. Next, 50 µL of chloroform/isoamyl alcohol (24:1) was added to the suspensions, and after vortexing, the mixture was centrifuged at 16 000 g for five minutes at 4°C. Then, 5 µL of the upper aqueous phase was used as a source of DNA template for the PCR reaction.

2.2.2 Amplification of the internally transcribed spacer (ITS) region and analysis of the amplified ribosomal DNA

The primers used for the amplification of the ITS region between the 16S and 23S rRNA genes were Forward primer: AGAGTTTGATCCTGGCTCAG and Reverese primer: CTTGTGCGGGCCCCCGTCAATTC. The

Polymerase Chain Reaction (PCR) was carried out by mixing 5 µL of each extracted DNA with 25 µL of 2X PCR Master kit (composition of 1X solution: 0.5 M Tris-HCl, 1.5 mM $MgCl_2$ – 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM dTTP and 0.04 Units/ul Taq), 1 µL Oligo forward (10 picomole/µL), 1 µL Oligo reverse (10 picomole/µL) and 18 µL Sterile deionized water.

The amplification was achieved by 40 PCR cycles. The Amplified product were examined using 1.5% (w/v) agarose gels in 0.5X TBE buffer at 75 V for 90 minutes with a DNA ladder. Product thus obtained was 875 bp.

2.3 Detection of Qualitative and Quantitative Lipolytic Activity

Purified isolates were checked for significant lipolytic activity by using agar spot method on selective Tributyrin Agar medium.

Titration method [13] was used to measure the amount of FFA released by the activity of crude lipase. The reaction mixture containing crude lipase preparation, tributyrin and phosphate buffer (0.2 M, pH 7.2) in 1:1:2 ratio respectively and incubated for 24 h at 37°C temperature. Each time free fatty acids content was determined using the formula (TN/PV)x103, where T is the net titration volume, N is the normality of the methanolic KOH, P is the proportion of the upper layer titrated, and V is the volume (in millilitres) of sample. One activity unit was defined as the amount of enzyme that produced 1 µmol butyric acid per hour under standard assay conditions.

Lipase activity was expressed in U /mg. Relative lipase activity was calculated by following formulae: C-E/E ×100

Where, C= Lipase activity observed from control, E= Lipase activity observed from preparations exposed different ranges of physical factors

3. RESULTS AND OBSERVATIONS

Results suggested that bacterial isolates purified from unfermented and fermented camel milk were identified as *Bacillus* (MRL a) and *Lactobacillus* (MRL b) respectively. Both are positive for Gram reaction and catalase negative. After culture based identification, representative isolates from each profile were analyzed for their 16S rDNA sequences. Homology searches of the sequences revealed (with 98 - 99% homology) that “MRL a” belongs to *Bacillus* and “MRL b” belongs to *Lactobacillus plantarum*.

These isolates also checked for the existence of liplytic activity. Both isolates, “MRL a” and “MRL b” showed significant lipolytic activity i.e. 12.66 mm; 30.37 FFAs/h//mg and 11.66 mm (Fig. 1; Table 1); 29.88 FFAs/h/mg respectively (Table 2).

4. DISCUSSION

Sedentary life style, stress and work load leads increase issues related to public health. One of major problem is enhanced level of serum lipid which directly affect heart health and consequently other organs. Need of hour is the

Table 1. Lipolytic activity of bacterial isolate

Source	Bacterial isolate	Zone of hydrolysis (in mm)			Mean± S.D.
		R1	R2	R3	
Fermented camel milk	*Lactobacillus plantarum*	12	11	12	11.66±0.57735
Unfermented camel milk	*Bacillus spp*	13	11	13	12.66±0.57735

Table 2. Quantitative estimation of Lipolytic activity by Titrimetric method

S. no	Source	Micromole of free fatty acids/hour				Specific lipase activity (Total activity/mg)
		R1	R2	R3	Mean±S.D.	
1.	Fermented camel milk	2.70	2.68	2.70	2.69±0.09	29.88
2.	Unfermented camel milk	2.45	2.46	2.39	2.43±0.003	30.37
3.	PC 1	1.083	1.041	1.083	1.069±0.019	13.362
4.	PC 2	0.833	0.958	0.833	0.874±0.058	10.925

PC1: Pseudomonas Lipase; PC 2: Pancreatic porcine lipase (Positive control)

discovery of natural ingredients or development of formulations which can be ingested without prescriptions. The present study deals with purification and identification of probiotic bacteria from camel milk. Camel milk is easily available in Rajasthan region and already known for its medicinal properties.

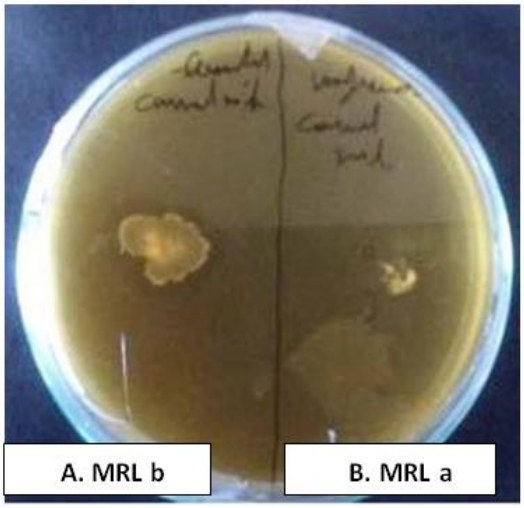

Fig. 1. Lipolytic activity of purified isolates
A. : Lactobacillus plantarum (MRLb)
B.: Bacillus (MRLa)

Results of this study showed that, in the screening of camel milk for the presence of Lactic acid bacteria community with lipolytic activity, Lactobacillus *plantarum* were dominant. It was found that the selected primers were able to amplify the ITS region between the 16S and 23S rRNA genes of Lactobacillus isolate from camel milk. This study revealed that identified Lactic acid bacteria, *Lactobacillus plantarum* showed significant lipolytic activity on tributyrin agar plate i.e. 11.66 mm and release 29.88 FFAs/h/mg. Consuming camel milk and its fermented products can help human health and protect the body against occurrences of food poisoning [14].

In another study on camel milk, Yateem et al. [15] showed the presence of *L. plantarum*, *L. pentosus* and *L. lactis* as probiotic LAB in raw camel milk. This study suggests that camel milk is a potential source for the probiotic LAB strains showing significant lipolytic activity.

After clinical trials these isolates can be used to enrich camel milk or can be used to develop fortified formulation which can be helpful for peoples suffering from high serum lipid level and their consequences.

5. CONCLUSION

Isolation of lipid degrading *Lactobacillus* bacteria from camel milk improves its therapeutic property. *Lactobacillus spp* purified from this sample showed significant lipase activity hence probiotic formulation developed from the same can help for those people suffering from complications associated with high lipid level.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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