

Research Paper

Characterization of Lactic acid bacteria from raw milk samples of Cow, Goat, Sheep, Camel and Buffalo with special elucidation to lactic acid production.

ABSTRACT:

Aims: Selective enumeration, isolation and characterization of lactic acid bacteria from different raw milk sources with special elucidation to lactic acid production

Study design: Serial dilution for strain isolation from raw milk samples to be done followed by complete morphological, biochemical and molecular characterization aiming to study the microbial behavior to ferment different sugar and producing lactic acid as end product.

Place and Duration of Study: Microbiology Biotechnology Laboratory, Tropilite Foods Pvt. Ltd. Gwalior (M.P)-INDIA from Sep 2012 to April 2013

Methodology: Milk samples from cow, sheep, goat, camel and buffalo were collected from the surrounding area of Gwalior district of Madhya Pradesh, India, from local milk suppliers. 13 potent strains in terms of lactic acid production were selected for analysis. Gram Staining, Catalase activity, Sugar fermentation, growth at high (45⁰ C) and low (10⁰ C) temperatures along with growth in different NaCl concentrations was observed with all isolates. Significant molecular characterization was done to determine the homology between different isolates of lactic acid bacteria

Results and Conclusion: Five potent lactic acid bacteria strains *Streptococcus thermophilus* from goat milk, *Lactococcus lactis* from buffalo milk, *Streptococcus gallolyticus* from camel milk, *Streptococcus thermophilus* from cow milk, *Lactobacillus delbrueckii* from sheep milk were identified capable of producing lactic acid in generous amount. Also all isolated strains from goat milk were found efficient in terms of lactic acid production when compared to other raw milk sources.

Key Words: Lactic acid bacteria, Goat milk, lactic acid, sugar fermentation

1. INTRODUCTION

Milk and milk products provide a wealth of nutrition benefits with their healthy contents along with the micro-flora these products carry. There are a variety of bacteria that can be present in milk. Among other differences, they vary in the temperatures at which they will grow optimally. Some are described as psychrophilic, which means that they grow best at cold temperatures, while others are severely retarded by being in the refrigerator and grow rapidly only at warmer temperatures. The microbiological quality of milk and milk products is influenced by the initial flora of raw milk. Among all the milk micro-flora, only lactic acid bacteria have the property to convert natural sugars into lactic acid by fermentation. When

camel milk samples are left to stand, acidity increases due to the presence of lactic acid bacteria [1], same is the result with cow, goat, sheep and buffalo milk. This acidity is due to the presence of lactic acid which was firstly discovered in sour milk in 1780 by a Swedish chemist, Carl Wilhelm Scheele, who initially considered it as milk component which was in 1857 corrected by Louis Pasteur as a fermentation end product [2]. The production of lactic acid has been used for a long time in food production (e.g., yogurt, cheese, sauerkraut, sausage,). Since the 1970s, the popularity of fermented foods such as kefir, kuniss, and tofu that were formally confined to certain ethnically oriented cuisines has greatly increased.

The lactic acid bacteria are a group of Gram positive bacteria. Other main characteristics include non-respiring, non-spore forming, cocci or rods, and produce lactic acid as the major end product from the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of sour dough bread, all fermented milks, cassava (to produce gari and fufu), and most fermented vegetables. The production of this fermented food is still largely a traditional art associated with poor hygiene, inconsistent quality presentation and short shelf life which is due to acidity development. But this acidity also plays a crucial role in majority of food fermentations by the impact of time duration and amount produced.

2. MATERIAL AND METHODS

2.1 Sample Collection: Milk samples from cow, sheep, goat, camel and buffalo were collected from the surrounding area of Gwalior district of Madhya Pradesh, India. The sources of samples were urban dairy farms and local milk suppliers. Raw milk samples were collected in sterile tubes and maintained in chilled condition (8-10° C) during their transport to Microbiology Biotechnology Laboratory, Tropilite Foods Pvt. Ltd. Gwalior, for further analysis and research.

2.2 Isolation of Lactic acid bacteria: Serial dilutions of all raw milk samples in 0.1% peptone saline were used for microbial isolation on MRS (DE MAN, ROGOSA and SHARPE) agar plates. Plates were incubated for 24 hours at 38° C followed by picking the distinguishable colonies by sterile loop. Isolation methods followed were similar to those recommended by Van den Berg et al [3]. With raw milk samples from cow, goat, sheep, camel and buffalo the isolates were divided into five groups from group I to group V starting from the sample of their origin respectively. All the isolates were further cultured to obtain purity. Purification of the isolates was confirmed by Gram Staining and pure isolated were maintained MRS agar plates at 4° C.

2.3 Culture Identification: Gram Staining, Catalase activity, Sugar fermentation and growth in 5% Nacl was observed with all isolates [4], which leads the research on a way from where only Gram positive and Catalase negative samples were shortlisted for further analysis [5]. Selected isolates were inoculated in MRS broth at pH 6.5 to check the maximum lactic acid productivity which was measured by pH droppage after incubation of 24 hours.

3. RESULTS AND DISCUSSION

3.1 Strain selection for Lactic acid production: Isolates with tendency to ferment sugar into lactic acid efficiently were further allowed to multiply individually and concentrations of lactic acid productivity was determined (Table 1) by using HPLC and the yield was calculated by the equation (1)

$$\text{Percentage yield} = \frac{\text{Grams of Lactic acid produced}}{\text{X 100}} \quad (1)$$

88 Grams of Total sugar consumed

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90 **Table 1. Percentage yield of lactic acid production from selected LAB isolates**

S. No.	Code of Isolate	Source of Isolate	Percentage yield of Lactic acid production
1	C1	RAW COW MILK	31.45
2	C2	RAW COW MILK	27.66
3	C7	RAW COW MILK	22.13
4	G1	RAW GOAT MILK	82.66
5	G7	RAW GOAT MILK	56.34
6	G22	RAW GOAT MILK	72.91
7	S2	RAW SHEEP MILK	12.22
8	S5	RAW SHEEP MILK	17.23
9	S16	RAW SHEEP MILK	21.36
10	CAM3	RAW CAMEL MILK	34.67
11	CAM7	RAW CAMEL MILK	33.21
12	B4	RAW BUFFALO MILK	57.61
13	B9	RAW BUFFALO MILK	54.29

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92 **3.2 Screening of Isolates:** A total of 13 Isolates with high percentage of lactic acid production were
 93 analyzed according to the methods described in Bergey's Manual [6]. The cultures were examined
 94 microscopically by staining and morphological characteristics were noted. Cultural, Physiological and
 95 biochemical characteristics specifically for lactic acid bacteria were also studied [7]. Growth
 96 characteristics at different temperatures were also monitored on daily basis at 10° C and 45° C in MRS
 97 medium over 7 days. Salt tolerance capability of the strains was accessed with 1.5%, 3% and 5%
 98 concentrations followed by casein hydrolysis and nitrate reductase test [Table 2(A), 2(B)].

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101 **Table 2(A). Morphological and Biochemical characterization of selected isolates**

Code of Isolates						
Characteristics	C1	C2	C7	G1	G7	G22
Cell Morphology	Cocci in chains	Short Rods	Cocci	Cocci in chains	Rods	Cocci

Gram staining	+	+	+	+	+	+
Spore Formation	-	-	-	-	-	-
Catalase Activity	-	-	-	-	-	-
Fermentation Type	Homo	Hetero	Homo	Hetero	Hetero	Homo
Glucose Fermentation	+	+	+	+	+	+
Nitrate Reduction	-	-	-	-	-	-
Casein Hydrolysis	+	+	+	+	+	+
Growth at 10° C	-	-	-	-	-	-
Growth at 45° C	+	-	-	+	-	+
Growth with 1.5% Nacl	+	-	+	-	-	+
Growth with 3% Nacl	-	-	-	-	-	-
Growth with 5% Nacl	-	-	-	-	-	-
At pH 4.2	-	-	-	+	-	-
Continued.						

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103 On the basis of lactic acid production from the isolated strains, only high acid production efficient strains
 104 were targeted with at least one strain from each milk source to obtain a comparative data. It was found
 105 interesting to observe that strains isolated from goat milk showed high acid productivity compared to other
 106 milk sources. Strain G1 and G22 specifically target lactose as sugar source to convert into lactic acid by
 107 fermentation. While as strain B4, B9 and G7 use sucrose to efficiently produce lactic acid at favorable
 108 temperatures.

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112 **Table 2(B). Morphological and Biochemical characterization of selected isolates**

Characteristics	Code of Isolates						
	S2	S5	S16	CAM3	CAM7	B4	B9
Cell Morphology	Cocci in chains	Long Rods	Rods	Cocci	Short Rods	Cocci in bunches	Rods

Gram staining	+	+	+	+	+	+	+
Spore Formation	-	-	-	-	-	-	-
Catalase Activity	-	-	-	-	-	-	-
Fermentation Type	Hetero	Hetero	Homo	Hetero	Hetero	Homo	Hetero
Glucose Fermentation	+	+	+	+	+	+	+
Nitrate Reduction	-	-	-	-	-	-	-
Casein Hydrolysis	+	+	+	+	+	+	+
Growth at 10	-	-	-	-	-	-	-
Growth at 45	+	-	-	+	-	-	+
Growth with 1.5% Nacl	+	-	+	-	+	-	-
Growth with 3% Nacl	-	-	-	-	-	-	-
Growth with 5% Nacl	-	-	-	-	-	-	-
At pH 4.2	-	-	-	+	+	-	-

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114 **Table 3. Sugar fermentation results from different selected isolates**

Isolate code	SUGAR FERMENTATION							
	Sucrose	Lactose	Maltose	Dextrose	Ribose	Sorbitol	Arabinose	Mannose
G1	+	+	+	+	+	+	+	+
B4	+	+	+	+		-	+	-
CAM3	+	+	+	+	-	-	-	+
C1	+	+	-	+	-	-	-	-
S16	+	+	+	+	-	+	-	-
+* indicates the specific sugar involved in maximum acid production for particular isolate								

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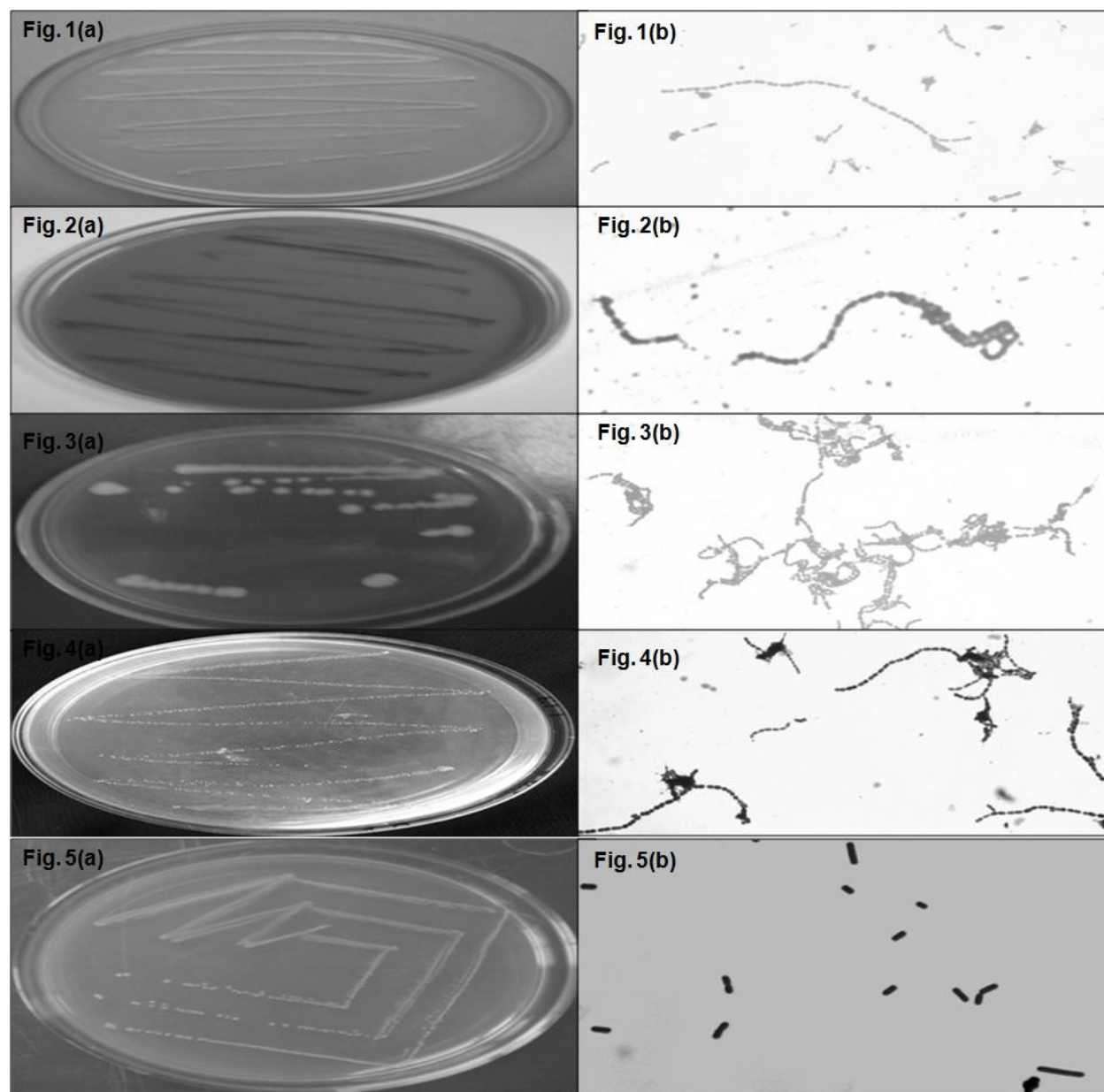


Figure 1(a), 2(a), 3(a), 4(a) and 5(a) presenting the growth of G1, B4, CAM3, C1 and S16 on MRS agar plates and on the right side is the morphological presentation of the same strains respectively in figures 1(b), 2(b), 3(b), 4(b) and 5(b).

All the phenotypic testing which involves identification on the basis of physiological, biochemical and chemotaxonomic methods [8] are sufficient for preliminary identification and the results concluded from this identification were found enough to classify all the five strains G1, B4, CAM3, C1 and S16 under lactic acid bacteria group. But the fact remains that four out of five strains were found *Streptococcus* with homology in their fermentation and other characteristics, which creates difficulty in identifying closely related type strains.

The distribution of lactic acid bacteria species are related to molecular studies, therefore it was found necessary to demonstrate significant molecular characterization. Despite the wide application of lactic

acid bacteria, they are currently differentiated by the determination of DNA-DNA similarity, their G+C content and, with more difficulty, by physiological characterization [9]. PCR amplification and subsequent sequencing of the 16S rDNA genes directly from selected isolates was done and the strains were identified on the basis of 16S rDNA homologies with entries in the GenBank NCBI databases (Table 4)

Table 4. Homology comparisons of isolated strains from database.

Isolate code	COMPARED WITH DATABASE	
	Strain homology	Percentage similarity
G1	<i>Streptococcus thermophilus</i> strain ATCC 19258 16S ribosomal RNA, complete sequence	96%
B4	<i>Lactococcus lactis</i> strain IMAU40136 16S ribosomal RNA gene, partial sequence	98%
CAM3	<i>Streptococcus gallolyticus</i> UCN34 strain UCN34 16S ribosomal RNA, complete sequence	94%
C1	<i>Streptococcus thermophilus</i> MN-ZLW-002 strain MN-ZLW-002 16S ribosomal RNA, complete sequence	95%
S16	<i>Lactobacillus delbrueckii</i> strain SDa4-56 16S ribosomal RNA gene, partial Sequence	93%

4. CONCLUSION

Lactic acid obtained by biotechnological process is preferred for industrial applications, especially, bioplastic industry. Lactic acid bacteria are good organisms for lactic acid fermentation. The intention of the present investigation was to study growth and multiplication of lactic acid producing micro-organisms from different milk sources and to observe the differences in between these organisms specifically for acid production from different environments, temperatures, and pH etc. Based on the current finding it was observed that the lactic acid bacteria strains isolated from goat milk are efficient in terms of lactic acid production in amassed quantities and fast acid producing also as compared to raw milk samples from cow, sheep, camel and buffalo. It was also observed that strains isolated from camel raw milk are comparatively more tolerant to acids.

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