



Probiotic Potentials and Biochemical Characterization of Lactic Acid Bacteria Isolated from Different Curd Samples

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Abstract

Curd have been well known source of *Lactibacillus*. In the present study, 11 and 14 bacterial isolates were obtained from curd of powdered and raw cow milk respectively. The bacterial isolates obtained were studied for their morphological, biochemical and physiological properties, probiotic potentials and also their antibiotic resistance. Based on the morphological and biochemical characteristic, 7 isolates from curd of cow milk were tentatively identified as *Lactibacillus* spp; 5 as *Leuconostoc* spp. and 1 *Streptococcus* spp; while 6 isolates from curd of powdered milk were tentatively identified as *Lactobacillus* spp, and 4 as *Leuconostoc* spp and 1 *Lactococcus* spp. Calorimetric estimation of both vitamin A and vitamin C content and titrimetric estimation of casein content of both curd sample showed good result. Total antioxidant capacity was determined to evaluate the total antioxidant potential of the sample material. Catalase activity was determined in terms of molar extinction co-efficient ($\text{Mm}^{-1}\text{cm}^{-1}$). The IAR analysis of the 14 isolates from curd of cow milk showed that the isolates CBL₂, CBL₄, CBL₇, CBL₉ and CBL₁₁ were resistant to all antibiotic materials and in case of curd from powdered milk all the isolates except PBI₁, PBI₆ and PBI₇ (against ampicillin) and PBI₆ (against *Streptomycin* spp.) were resistant to all antibiotic material tested. Isolated strains of *Lactobacillus* spp. showed satisfactory probiotic potentials with reference strain.

Keywords

Bacterial isolates, Casein content, Catalase activity, Antioxidant, Antibiotic resistance.

INTRODUCTION

Microorganisms are most important in dairy products. One of the very important groups of acid producing bacteria in the food industry is the lactic acid bacteria, which are used in making starter culture for dairy products. Lactic acid bacteria play an important role in fermentation process and have a great influence on the quality and preservation of the end products. Lactic acid bacteria are characterised as gram positive, usually non motile, non-sporulating. The preservative effect of lactic acid bacteria during the manufacture and subsequent storage of fermented foods is mainly due to acidic conditions that they create and converting carbohydrates to organic acids (lactic acid and acetic acid) in the food during their development. Among all lactic acid bacteria, the genus *Lactobacillus* has many beneficial characteristics which make it useful for the industrial application¹. *Lactobacillus* can produce a variety of substances such as lactic acid, ethanol, formic acid, acetone, hydrogen peroxide diacetyl etc, which has gaining importance because of their potential applications in food industries as texturizers, viscosifiers, emulsifiers and syneresis lowering agents, for their pseudoplastic behaviour and water binding capacity. In raw milk *Lactobacillus* are naturally present also ingestion of live cells of certain strains of *Lactobacillus* in adequate amounts in raw milk believed to confer several beneficial physiological effects on the host among these is the maintenance of healthy and equilibrated intestinal flora and resistance to intestinal infections².

MATERIALS AND METHODS

Collection of Milk Samples:

Both the powdered and cow milk sample were collected from the local market of Mangaldai and curd were prepared, then stored at 4°C in refrigeration for further use.

Isolation of bacteria:

Fourteen and eleven bacterial isolates were obtained from the prepared sample and maintained as culture bank in the IBT hub Mangaldai College. Isolation was made following serial dilution agar plate method using nutrient agar media³.

Identification and Characterization of the Bacterial Isolates:

Morphological Characterization:

All the isolates were examined for their colony morphology, pigmentation, cell shape and gram reaction as per the standard procedures given by Bartholomew *et al.*⁴.

Biochemical Characterization:

Biochemical characterization of all the isolates i.e., starch hydrolysis, IMViC Test, Citrate utilization test, hydrogen sulphide test, catalase test, urease test, acid and gas production (carbohydrate fermentation) was done as per the procedures outlined by Cappuccino and Sherman⁵.

Probiotic property analysis of curd:

The estimation of vitamin A and vitamin C was done using the method described by Bayfield and Cole⁶ respectively.

The quantification of Casein content in the prepared curd material was evaluated titrimetrically and expressed in µg/g of the sample. This method was followed by McDowell and McDowell⁷.

Antioxidant activity:

The DPPH free radicals scavenging activity of the sample was done using the method described by Stojkovic *et al.*⁸

Evaluation of catalase activity:

Catalase activity was determined following the method of Aebi⁹.

Intrinsic Antimicrobial Activity:

Antimicrobial activity was determined by using the method described by Nawaz *et al.*¹⁰.

RESULT AND DISCUSSION:

From Table 1a, it is found that all isolates from curd of powdered milk non-spreading colony, rod shaped cell except PBI₈, gram positive in case of PBI₁, PBI₂, PBI₃, PBI₆, PBI₇, PBI₁₁ and gram negative in case of PBI₄, PBI₅, PBI₈, PBI₉ and PBI₁₀. Table 1b showed that all the isolates from curd of cow milk were round shaped, non-spreading colony, rod shaped cell except CBI₈ and CBI₁₃, gram positive in case of CBI₁, CBI₂, CBI₃, CBI₆, CBI₇, CBI₁₁ & CBI₁₂ and gram negative in case of CBI₄, CBI₅, CBI₈, CBI₉, CBI₁₀, CBI₁₃ & CBI₁₄. From table 2, we found that both curd sample have good amount of vitamin A, vitamin C and casein content. Biochemical characterization of the bacterial isolated of both curd samples were presented in Table 3a & 3b & identification of the isolated up to the genus level was carried out. 7 isolates from curd of cow milk were identified as *Lactobacillus* spp., 6 as *Leuconostoc* spp. and 1 as *Streptococcus* spp., while 6 isolates from curd of powdered milk were identified as *Lactobacillus* spp., 4 as *Leuconostoc* spp. and 1 *Lactococcus* spp. Intrinsic antibiotic resistance of the bacterial isolates from both the curd sample were presented in Table 4a & 4b; the isolates CBL₂, CBL₄, CBL₇, CBL₉ and CBL₁₁ were resistant to all antibiotic materials and in case of curd from powdered milk all the isolates except PBI₁, PBI₆, and PBI₇ (against ampicillin) and PBI₆ (against

Streptomyces spp.) were resistant to all antibiotic material tested. Antioxidant and catalase activity of both the card sample in table 5 showed good amount.

Table 1a: Morphological characteristics of the bacterial isolates from curd of powdered milk.

Sl. No.	Isolates	Colony Morphology			Cell Shape	Gram Reaction
		Colour	Shape	Nature		
1	PBI ₁	Pale-Yellow	Round	Non spreading	Rod	Positive
2	PBI ₂	Cream	Round	Non spreading	Rod	Positive
3	PBI ₃	Yellow	Round	Non spreading	Rod	Positive
4	PBI ₄	Yellow	Round	Non spreading	Rod	Negative
5	PBI ₅	Yellow	Round	Non spreading	Rod	Negative
6	PBI ₆	Pale-Yellow	Round	Non spreading	Rod	Positive
7	PBI ₇	Yellow	Round	Non spreading	Rod	Positive
8	PBI ₈	Cream	Round	Non spreading	Round	Negative
9	PBI ₉	Cream	Round	Non spreading	Rod	Negative
10	PBI ₁₀	Yellow	Round	Non spreading	Rod	Negative
11	PBI ₁₁	Yellow	Round	Non spreading	Rod	Positive

PBI = Powdered Bacterial Isolates (B₁-B₁₁) "+" = Positive "-" =Negative

Table1b: Morphological characteristics of the bacterial isolates from curd of cow milk.

Sl. No.	Isolates	Colony Morphology			Cell Shape	Gram Reaction
		Colour	Shape	Nature		
1	CBI ₁	Pale-Yellow	Round	Non spreading	Rod	Positive
2	CBI ₂	Cream	Round	Non spreading	Rod	Positive
3	CBI ₃	Yellow	Round	Non spreading	Rod	Positive
4	CBI ₄	Yellow	Round	Non spreading	Rod	Negative
5	CBI ₅	Yellow	Round	Non spreading	Rod	Negative
6	CBI ₆	Pale-Yellow	Round	Non spreading	Rod	Positive
7	CBI ₇	Yellow	Round	Non spreading	Rod	Positive
8	CBI ₈	Cream	Round	Non spreading	Round	Negative
9	CBI ₉	Cream	Round	Non spreading	Rod	Negative
10	CBI ₁₀	Yellow	Round	Non spreading	Rod	Negative
11	CBI ₁₁	Yellow	Round	Non spreading	Rod	Positive
12	CBI ₁₂	Pale-Yellow	Round	Non spreading	Rod	Positive
13	CBI ₁₃	Yellow	Round	Non spreading	Round	Negative
14	CBI ₁₄	Yellow	Round	Non spreading	Rod	Negative

CBI = Cow Bacterial Isolates (B₁-B₁₄) "+" = Positive "-" =Negative

Table 2: Vitamin-A, Vitamin-C and casein content in the curd of powdered milk and cow milk

Sample	Parameter		
	Vitamin-A (µg/g of the sample)	Vitamin-C (µg/g of the sample)	Casein (µg/g of the sample)
Curd of powdered milk	0.70	0.015	0.75
Curd of cow milk	0.50	0.025	0.525

Table 3a: Biochemical characterization of bacterial isolates from curd of powdered milk

Sl. No.	Organism Code	Starch hydrolysis	Casein hydrolysis	Triple Sugar Iron Agar Test/ H ₂ S	Carbohydrate Fermentation			MR	VP	Citrate Utilization	Urease Test	Indole production	Organism
					Lactose	Dextrose	Sucrose						
1	PBI ₁	+	+	+	+	+	+	+	-	-	+	-	<i>Lactobacillus</i>
2	PBI ₂	+	+	+	+	+	+	-	-	-	+	-	<i>Lactobacillus</i>
3	PBI ₃	+	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus</i>
4	PBI ₄	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
5	PBI ₅	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
6	PBI ₆	+	+	+	+	+	+	+	-	-	-	-	<i>Lactobacillus</i>
7	PBI ₇	+	+	+	+	-	+	+	-	-	+	+	<i>Lactobacillus</i>
8	PBI ₈	-	+	-	+	+	+	+	-	-	+	+	<i>Lactococcus spp.</i>
9	PBI ₉	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
10	PBI ₁₀	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
11	PBI ₁₁	-	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus</i>

PBI = Powdered Bacterial Isolates (B₁-B₁₁) "+" = Positive "-" = Negative, MR- Methyl Red, VP - Vogesproskauer.

Table 3b: Biochemical characterization of bacterial isolates from curd of cow milk

Sl. No.	Organism Code	Starch hydrolysis	Casein hydrolysis	Triple Sugar Iron Agar Test/ H ₂ S	Carbohydrate Fermentation			MR	VP	Citrate Utilization	Urease Test	Indole production	Organism
					Lactose	Dextrose	Sucrose						
1	CBI ₁	+	+	+	+	+	+	+	-	-	+	-	<i>Lactobacillus spp.</i>
2	CBI ₂	+	+	+	+	+	+	-	-	-	+	-	<i>Lactobacillus spp.</i>
3	CBI ₃	+	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
4	CBI ₄	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
5	CBI ₅	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
6	CBI ₆	+	+	+	+	+	+	+	-	-	-	-	<i>Lactobacillus spp.</i>
7	CBI ₇	+	+	+	+	-	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
8	CBI ₈	-	+	-	+	+	+	+	-	-	+	+	<i>Lactococcus spp.</i>
9	CBI ₉	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
10	CBI ₁₀	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
11	CBI ₁₁	-	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
12	CBI ₁₂	+	+	+	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
13	CBI ₁₃	-	+	-	+	+	+	+	-	-	+	+	<i>Streptococcus spp.</i>
14	CBI ₁₄	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>

CBI = Curd Bacterial Isolates (B₁-B₁₄) "+" = Positive "-" = Negative, MR - Methyl Red, VP - Vogesproskauer.

Table 4a: Intrinsic antibiotic resistance of the bacterial isolates from curd of powdered milk

Sl. No.	Isolates	Zone of inhibition against antibiotic material (in mm)				
		Amp ₂₅	Clm ₅	Neo ₁₀	Str ₁₀₀	Tet ₂₀
1	PBI-1	10.0	-	-	-	-
2	PBI-2	-	-	-	-	-
3	PBI-3	-	-	-	-	-
4	PBI-4	-	-	-	-	-
5	PBI-5	-	-	-	-	-
6	PBI-6	9.0	-	-	9	-
7	PBI-7	9.0	-	-	-	-
8	PBI-8	-	-	-	-	-
9	PBI-9	-	-	-	-	-
10	PBI-10	-	-	-	-	-
11	PBI-11	-	-	-	-	-

PBI-1- PBI-11: Powdered bacterial isolates, Amp₂₅: Ampicilin 25 µg/mL, Clm₅ Chloramphenicol 5 µg/mL, Neo₁₀: Neomycin 10 µg/mL, Tet₂₀: Tetracycline 20 µg/mL, Str₁₀₀: Streptomycin 100 µg/mL, Zone of inhibition including 5mm well diameter

Table 4b: Intrinsic antibiotic resistance of the bacterial isolates from curd of cow milk

Sl. No.	Isolates	Zone of inhibition against antibiotic material (in mm)				
		Amp ₂₅	Clm ₅	Neo ₁₀	Str ₁₀₀	Tet ₂₀
1	CBI-1	-	-	-	9.0	-
2	CBI-2	-	-	-	-	-
3	CBI-3	11.0	-	-	-	-
4	CBI-4	-	-	-	-	-
5	CBI-5	-	-	-	11.00	-
6	CBI-6	9.0	-	-	-	-
7	CBI-7	-	-	-	-	-
8	CBI-8	9.0	8.0	-	8.0	-
9	CBI-9	-	-	-	-	-
10	CBI-10	10.0	-	-	-	-
11	CBI-11	-	-	-	-	-
12	CBI-12	-	-	-	9.0	-
13	CBI-13	9.0	-	-	-	-
14	CBI-14	-	-	-	8.0	-

CBI-1- CBI-14: Cow Bacterial Isolates, Amp₂₅: Ampicilin 25 µg/mL, Clm₅ Chloramphenicol 5 µg/mL, Neo₁₀: Neomycin 10 µg/mL, Tet₂₀: Tetracycline 20 µg/mL, Str₁₀₀: Streptomycin 100 µg/mL, Zone of inhibition including 5mm well diameter

Table 5: Antioxidant and Catalase activity (mM⁻¹cm⁻¹) of powdered milk and cow milk

Sample	Parameter	
	Antioxidant activity (%)	Catalase activity (%)
Curd of powdered milk	71.15 ±0.05	0.004±0.001
Curd of cow milk	61.15 ±0.05	0.005±0.001

CONCLUSION:

The result of this study indicate that both powdered and row cow milk exhibit a wide diversity of lactic acid producing bacteria occurring naturally in milk. From the present study, it is proved that *Lactobacillus* is predominant in card of both cow and powdered milk, since some strains of lactic acid bacteria possess potential probiotic properties,

further work in respect to other features of these strains is needed which is going on.

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