



# 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 contain 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 ( $Mm^{-1}cm^{-1}$ ). The IAR analysis of the 14 isolates from curd of cow milk showed that the isolates CBL<sub>2</sub>, CBL<sub>4</sub>, CBL<sub>7</sub>, CBL<sub>9</sub> and CBL<sub>11</sub> were resistant to all antibiotic materials and in case of curd from powdered milk all the isolates except PBI<sub>1</sub>, PBI<sub>6</sub> and PBI<sub>7</sub> (against ampicillin) and PBI<sub>6</sub> (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.*

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## 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-sporeforming. 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 application<sup>1</sup>. *Lactobacillus* can produce a variety of substances such as lactic acid, ethanol, formic acid, acetone, hydrogen peroxide diacetyletc, 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 infections<sup>2</sup>.

## 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 media<sup>3</sup>.

### 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<sup>4</sup>.

### 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 Sherman<sup>5</sup>.

### Probiotic property analysis of curd:

The estimation of vitamin A and vitamin C was done using the method described by Bayfield and Cole<sup>6</sup> 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 McDowall and McDowell<sup>7</sup>.

### Antioxidant activity:

The DPPH free radicals scavenging activity of the sample was done using the method described spp. Stojkovic et al<sup>8</sup>

### Evaluation of catalase activity:

Catalase activity was determined following the method of Aebi<sup>9</sup>.

### Intrinsic Antimicrobial Activity:

Antimicrobial activity was determined by using the method described by Nawaz et al<sup>10</sup>.

## RESULT AND DISCUSSION:

From Table 1a, it is found that all isolates from curd of powdered milk non-spreading colony, rod shaped cell except PBI<sub>8</sub>, gram positive in case of PBI<sub>1</sub>, PBI<sub>2</sub>, PBI<sub>3</sub>, PBI<sub>6</sub>, PBI<sub>7</sub>, PBI<sub>11</sub> and gram negative in case of PBI<sub>4</sub>, PBI<sub>5</sub>, PBI<sub>8</sub>, PBI<sub>9</sub> and PBI<sub>10</sub>. Table 1b showed that all the isolates from curd of cow milk were round shaped, non-spreading colony, rod shaped cell except CBI<sub>8</sub> and CBI<sub>13</sub>, gram positive in case of CBI<sub>1</sub>, CBI<sub>2</sub>, CBI<sub>3</sub>, CBI<sub>6</sub>, CBI<sub>7</sub>, CBI<sub>11</sub> & CBI<sub>12</sub> and gram negative in case of CBI<sub>4</sub>, CBI<sub>5</sub>, CBI<sub>8</sub>, CBI<sub>9</sub>, CBI<sub>10</sub>, CBI<sub>13</sub> & CBI<sub>14</sub>. 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 card sample were presented in Table 4a & 4b ; the isolates CBL<sub>2</sub>, CBL<sub>4</sub>, CBL<sub>7</sub>, CBL<sub>9</sub> and CBL<sub>11</sub> were resistant to all antibiotic materials and in case of curd from powdered milk all the isolates except PBI<sub>1</sub>, PBI<sub>6</sub>, and PBI<sub>7</sub> (against ampicillin) and PBI<sub>6</sub> (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 <sub>1</sub>	Pale-Yellow	Round	Non spreading	Rod	Positive
2	PBI <sub>2</sub>	Cream	Round	Non spreading	Rod	Positive
3	PBI <sub>3</sub>	Yellow	Round	Non spreading	Rod	Positive
4	PBI <sub>4</sub>	Yellow	Round	Non spreading	Rod	Negative
5	PBI <sub>5</sub>	Yellow	Round	Non spreading	Rod	Negative
6	PBI <sub>6</sub>	Pale-Yellow	Round	Non spreading	Rod	Positive
7	PBI <sub>7</sub>	Yellow	Round	Non spreading	Rod	Positive
8	PBI <sub>8</sub>	Cream	Round	Non spreading	Round	Negative
9	PBI <sub>9</sub>	Cream	Round	Non spreading	Rod	Negative
10	PBI <sub>10</sub>	Yellow	Round	Non spreading	Rod	Negative
11	PBI <sub>11</sub>	Yellow	Round	Non spreading	Rod	Positive

PBI = Powdered Bacterial Isolates (B<sub>1</sub>-B<sub>11</sub>) "+" = Positive "-" = Negative

**Table 1b: 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 <sub>1</sub>	Pale-Yellow	Round	Non spreading	Rod	Positive
2	CBI <sub>2</sub>	Cream	Round	Non spreading	Rod	Positive
3	CBI <sub>3</sub>	Yellow	Round	Non spreading	Rod	Positive
4	CBI <sub>4</sub>	Yellow	Round	Non spreading	Rod	Negative
5	CBI <sub>5</sub>	Yellow	Round	Non spreading	Rod	Negative
6	CBI <sub>6</sub>	Pale-Yellow	Round	Non spreading	Rod	Positive
7	CBI <sub>7</sub>	Yellow	Round	Non spreading	Rod	Positive
8	CBI <sub>8</sub>	Cream	Round	Non spreading	Round	Negative
9	CBI <sub>9</sub>	Cream	Round	Non spreading	Rod	Negative
10	CBI <sub>10</sub>	Yellow	Round	Non spreading	Rod	Negative
11	CBI <sub>11</sub>	Yellow	Round	Non spreading	Rod	Positive
12	CBI <sub>12</sub>	Pale-Yellow	Round	Non spreading	Rod	Positive
13	CBI <sub>13</sub>	Yellow	Round	Non spreading	Round	Negative
14	CBI <sub>14</sub>	Yellow	Round	Non spreading	Rod	Negative

CBI = Cow Bacterial Isolates (B<sub>1</sub>-B<sub>14</sub>) "+" = Positive "-" = Negative

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

Sample	Parameter		
	Vitamin-A ( $\mu$ g/g of the sample)	Vitamin-C ( $\mu$ g/g of the sample)	Casein ( $\mu$ g/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 <sub>2</sub> S	Carbohydrate Fermentation			MR	VP	Citrate Utilization	Urease Test	Indole production	Organism
					Lactose	Dextrose	Sucrose						
1	PBI <sub>1</sub>	+	+	+	+	+	+	+	-	-	+	-	<i>Lactobacillus</i>
2	PBI <sub>2</sub>	+	+	+	+	+	+	-	-	-	+	-	<i>Lactobacillus</i>
3	PBI <sub>3</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus</i>
4	PBI <sub>4</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
5	PBI <sub>5</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
6	PBI <sub>6</sub>	+	+	+	+	+	+	+	-	-	-	-	<i>Lactobacillus</i>
7	PBI <sub>7</sub>	+	+	+	+	-	+	+	-	-	+	+	<i>Lactobacillus</i>
8	PBI <sub>8</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Lactococcus spp.</i>
9	PBI <sub>9</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
10	PBI <sub>10</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
11	PBI <sub>11</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus</i>

PBI = Powdered Bacterial Isolates (B<sub>1</sub>-B<sub>11</sub>) "+" = 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 <sub>2</sub> S	Carbohydrate Fermentation			MR	VP	Citrate Utilization	Urease Test	Indole production	Organism
					Lactose	Dextrose	Sucrose						
1	CBI <sub>1</sub>	+	+	+	+	+	+	+	-	-	+	-	<i>Lactobacillus spp.</i>
2	CBI <sub>2</sub>	+	+	+	+	+	+	-	-	-	+	-	<i>Lactobacillus spp.</i>
3	CBI <sub>3</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
4	CBI <sub>4</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
5	CBI <sub>5</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
6	CBI <sub>6</sub>	+	+	+	+	+	+	+	-	-	-	-	<i>Lactobacillus spp.</i>
7	CBI <sub>7</sub>	+	+	+	+	-	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
8	CBI <sub>8</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Lactococcus spp.</i>
9	CBI <sub>9</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
10	CBI <sub>10</sub>	+	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
11	CBI <sub>11</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Lactobacillus spp.</i>
12	CBI <sub>12</sub>	+	+	+	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>
13	CBI <sub>13</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Streptococcus spp.</i>
14	CBI <sub>14</sub>	-	+	-	+	+	+	+	-	-	+	+	<i>Leuconostoc spp.</i>

CBI = Curd Bacterial Isolates (B<sub>1</sub>-B<sub>14</sub>) "+" = 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 <sub>25</sub>	Clm <sub>5</sub>	Neo <sub>10</sub>	Str <sub>100</sub>	Tet <sub>20</sub>
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, Amp<sub>25</sub>: Ampicillin 25 µg/mL, Clm<sub>5</sub>: Chloramphenicol 5 µg/mL, Neo<sub>10</sub>: Neomycin 10 µg/mL, Tet<sub>20</sub>: Tetracycline 20 µg/mL, Str<sub>100</sub>: 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 <sub>25</sub>	Clm <sub>5</sub>	Neo <sub>10</sub>	Str <sub>100</sub>	Tet <sub>20</sub>
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, Amp<sub>25</sub>: Ampicillin 25 µg/mL, Clm<sub>5</sub>: Chloramphenicol 5 µg/mL, Neo<sub>10</sub>: Neomycin 10 µg/mL, Tet<sub>20</sub>: Tetracycline 20 µg/mL, Str<sub>100</sub>: Streptomycin 100 µg/mL, Zone of inhibition including 5mm well diameter

**Table 5: Antioxidant and Catalase activity (mM<sup>-1</sup>cm<sup>-1</sup>) 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 raw 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 curd 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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