Occurrence of Lactobacilli in the Traditional Preparation of Curd

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Abstract

Curd is a dairy product produced via way of means of coagulating milk in a process known as curdling. The objective of this current study was to prepare curd traditionally in the laboratory and identify Lactobacilli species with potential probiotic activities. Cow and goat milk was collected, and curd was prepared by adding lime and starter culture in different amounts. Among prepared curd samples, best quality curd was selected, and from the selected curd samples, lactic acid bacteria were isolated and subsequent subculturing was carried out, and ten different Lactobacilli isolates were obtained and identified based on morphological, physiological and biochemical characteristics.

Isolates were subjected to test the antagonistic effect among the Lactobacilli isolates and against selected bacterial strains by standard agar well diffusion method. Antibiotic sensitivity test by disc method was carried out by using antibiotics and the production of lactic acid by titration method.

Ten different Lactobacilli isolates were classified into three different genera *Lactobacillus* sp., *Pediococcus* sp. and *Leuconostoc* sp. Growth of all Lactobacilli was observed under acidic and neutral pH. Lactic acid bacteria exhibited different tolerant levels to bile salt. No antagonistic effect was observed among the Lactobacilli isolates. But 80% of lactobacilli isolates had antibacterial activity on selected bacterial species. The growth of *Bacillus* sp. was predominantly inhibited by Lactic acid bacterial isolates (90%), *Pseudomonas aeruginosa* (30%), *Proteus* sp. and *Staphylococcus aureus* (50%) and *E.coli* (30%). *Lactobacillus* sp. (CL2) predominantly inhibited all tested bacteria (100%). Growth of the Lactobacilli isolates was inhibited by tested antibiotics except for *Lactobacillus sp*. (CC3) was resistant to bacitracin only. All of the Lactic acid bacterial isolates produced a considerable amount of lactic acid. Under the controlled conditions, the Lactobacillus sp. (GL1) produced a significantly high level of lactic acid (0. 315%), whereas *Lactobacillus* sp. (CL3) produced less amount of lactic acid (0.099%).

Keywords: Curd, Lactic acid bacteria, Antagonistic effect, Antibacterial sensitivity, Lactic acid

1. Introduction

Food preservation has been largely influenced by the use of microorganisms and their products within the last few decades. Lactic acid bacteria (LAB) intend to be an appropriate candidate due to numerous antimicrobial, bio-therapeutic and preservation products**.**

The native food varieties have been prepared by conventional techniques, and technology is being moved from one generation to another (Katawal et al., 2008). Fermentation is the oldest procedure experienced by human for the change of milk into products with an extended shelf life (Tamime et al., 1999).

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Curd is a fermented milk product produced from boiled or pasteurized milk by souring with microorganisms, especially by harmless Lactic acid bacterial cultures. The curd is a dairy product received with the aid of using coagulating milk in a process called 'curdling'. Coagulation can be done by the addition of rennet or any edible acidic ingredients such as lemon juice or vinegar and then letting it coagulate. After the curd formation remaining liquid, which contains whey proteins, is known as 'whey'.

Traditionally curd is a naturally fermented milk product obtained from boiled cow, buffalo or goat milk and soured using mixed lactic cultures. Unlike the curd crafted from cow milk, the curd from goat milk has a considerably better percentage of short-chain fatty acids in particular capric, caproic and caprylic acids and with smaller fats globules which makes curd more easily digestible (Mohan et al., 1985) and also not produce any allergic reactions in children(Ali et al., 2002).

The curd forms the richest source of "probiotics". Probiotics offer useful and healthy microflora to the alimentary canal through food without any hazard of adverse effects (Patel et al., 2014). One such example of beneficial microorganisms in the order Lactobacillales, which includes gram-positive, *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus* (Leroy et al., 2004).

Both the curd and yoghurt dairy products are produced by the fermentation of milk. Hence the key difference is that the yoghurt is produced by fermenting milk using bacteria known as "Yoghurt cultures", while the curd is produced by curdling with an edible acidic ingredient such as lemon juice or vinegar and then draining off the liquid portion. Further, the yoghurt is more acidic with the addition of artificial sweeteners and flavours at the same time curd is relatively less acidic and added with natural flavours. The starter cultures used in curd and yoghurt production are entirely different, which include *Lactobacillus bulgaricus,* and *Streptococcus thermophilus* in the production of yoghurt, whereas *Lactococcus lactis, Streptococcus thermophilus* and *Lactobacillus bulgaricus* curd production.

The contamination of yeast and mould during traditional curd preparation leads to spoilage in curd. , The low acidity of the product and heat used during the process, are sufficient barriers to pathogen growth in curd/yoghurt. If the milk contacts yeast and mould in the growth phase of the curd making, conditions become good for the growth of yeast and mould in the product (Leroy et al., 2004).Yeast and mould contamination from the air around the containers that hold the milk. In order to make sure the safety and quality of cultured curd or yoghurt food, safety testing must be carried out at numerous stages throughout the production process.

These probiotic bacteria improve the gut microbiota balance, and the protection against pathogens. These can include the synthesis of antimicrobial substances (e.g., organic acid, hydrogen peroxide and bacteriocins), which inhibit the growth of harmful microorganisms. Such bacteria can inhibit a large number of enteric and urinary pathogenic bacteria through antagonistic activity (Hutt et al., 2006).

The objective of this study was to prepare curd traditionally in the laboratory and identify Lactobacilli species with potential probiotic activities.

2. Methods and materials

2.1. Preparation of curd by the traditional method

2.1.1. Collection of samples

Two different types of milk (cow, and goat) were collected from the milk collecting centre in Jaffna.

2.1.2. Preparation of curd

The curd was prepared traditionally in the laboratory – by using 50ml of milk. Milk was boiled and allowed to cool; different volumes of lime (2-10ml) and different amounts of starter culture (2-10g)

were added separately and then incubated at room temperature for 24h. Then the quality of the curd was determined based on whey content, texture and colour.

2.2. Isolation of Lactic acid bacteria from prepared curd

Lactic acid bacteria strains were isolated from selected curd by weighing 1g of sample diluted with 10 ml sterile distilled water. This sample was mixed well with sterile water using a magnetic stirrer, and then the serial dilution technique was carried out and plated out on MRS agar medium by spread plate technique.

The plates were incubated for 48 hours at 37^{o}C. The different characteristics of lactic acid bacteria colonies growing over the incubated plates were picked up carefully and streaked on the MRS agar medium separately to obtain pure cultures. Pure culture colonies were transferred to MRS agar slants separately and maintained in the refrigerator at 4 ^{o}C, for further studies, and each isolate was assigned a code number.

2.3. Characterization of Lactic acid bacteria

2.3.1. Morphological characterization of bacterial isolates

The isolates were subjected to morphological studies by using cell characters and colony characters.

2.3.2. Biochemical characters

The isolates were subjected to biochemical characteristics such as catalase test, carbohydrate fermentation test (lactose, sucrose, glucose, fructose, galactose, maltose, and mannitol), hydrolysis test (starch, gelatin), nitrate reduction test, and indole test.

Catalase test: A loopful of bacterial culture was inoculated in the slide containing 3% hydrogen peroxide solution. A positive reaction was indicated by the formation of effervescence or appearance of bubbles due to the breaking down of hydrogen peroxide into oxygen and water.

Carbohydrate fermentation test: 1% Bacteriological peptone, 0.1% phenol red, 0.1% NaCl and 1% lactose were dissolved in distilled water, and then pH was adjusted to 7.4 by using HCl and NaOH. 10ml of the broth was dispensed into test tubes with Durham's tubes. This setup was autoclaved at 121 ^{o}C, and 15 lbs/inch2 pressure for 15 minutes. A loop full of each isolated culture was transferred into the medium separately. A control was also maintained. Then tubes were incubated at 37^{o}Cfor 24 hours. A positive result indicates the acid and gas production, and a negative result indicates the absence of acid and gas production. The above procedure was repeated to other sugars like sucrose, glucose, fructose, galactose, maltose, and mannitol.

Hydrolysis of starch: 2% Starch was dissolved with nutrient agar by a magnetic stirrer. This set-up was autoclaved at 121 ^{o}C, and 15 lbs/inch2 pressure for 15 minutes. The medium was dispensed into sterile Petri dishes and allowed to settle. A loop full of each isolated culture was transferred by streaking on the above medium separately. A control was also maintained. Then plates were incubated at 37 ^{o}C for 24 hours. After the incubation plates were flooded with iodine solution (I2/KI), a clear zone around the streaked area in the plates where the organism utilized the starch medium was recorded as positive.

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Hydrolysis of gelatin: 2% Nutrient gelatin readymade medium was dissolved well in distilled water by heating using a magnetic stirrer. 10ml of the medium was transferred into the test tubes. This set-up was autoclaved at 121 ^{o}C and 15 lbs/inch^2 pressure for 15 minutes. A loop full of each isolated culture was inoculated into a nutrient gelatin medium by the stabbing method. A control was also maintained. All tubes were incubated at 37^{o}C for 24 hours and stored in a refrigerator for 10 to 15 minutes. Liquefaction of medium along the stabbed line was confirmed as a positive result.

Nitrate reduction test: Potassium nitrate and nutrient broth were dissolved in distilled water, and 10ml of the medium was distributed into each test tube containing Durham's tubes, and tubes were plugged with cotton wool and these tubes were autoclaved at 121^{o}C and 15 lbs/inch2 pressure for 15 minutes. A loopful of pure isolated culture was inoculated into tubes containing nitrate broth with Durham's tubes. These tubes were incubated at 37^{o}C for 24 hours. One tube was kept as control. After incubation, 0.5 ml of sulfanilic acid and naphthylamine were added. The colour change from yellow to red and gas production indicates a positive result. The initial color of the control tube is yellow.

Indole test: 3% Tryptophan broth was dissolved well in distilled water by heating. 5 ml of the medium was transferred into the McCartney bottles. These setups were autoclaved at 121 ^{o}C and 15 lbs/inch2 pressure for 15 minutes. A loop full of each isolated culture was inoculated into tryptophan broth. Control was also maintained. All tubes were incubated at 37^{o}C for 24 hours. Then Kovac's reagent was added to the broth. The development of a pink colour ring was considered a positive result.

2.3.3. Physiological characteristics

The isolates were subjected to physiological characteristics such as NaCl tolerance test, pH tolerance test, bile tolerance test and motility test.

NaCl tolerance test: 1.3g Nutrient broth was dissolved in 100ml distilled water. Then 3g of sodium chloride was added into the above medium (3% NaCl). Then 10ml of saline nutrient broth was poured into test tubes separately and plugged with cotton wool. After that, those tubes were autoclaved at 121^{o}C and 15 lbs/inch^2 pressure for 15 minutes. The concentration of different pure culture isolates was standardized by using Mc Farland standard (0.5M). 1ml of inocula was transferred into the sterile NaCl broth separately, and this set-up was incubated at 37^{o}C for 24 hours. After the incubation above tubes were observed for bacterial growth. The positive result for the growth was indicated by the turbidity of the medium. The above procedure was repeated to other NaCl concentrations of 4%, 5%, 6%, and 7%.

Bile tolerance test: 2.8g Nutrient agar was dissolved in 100ml distilled water. 0.3g of bile salt was added to the above medium in the Duran bottle (0.3% bile salt). This was autoclaved at 121^{o}C and 15 lbs/inch^2 pressure for 15 minutes. After sterilization medium was poured into sterile petridishes. The concentration of the different pure culture isolates was standardized by using Mc Farland standard (0.5M). 1ml of inoculum was inoculated in the centre of the medium. The spread plate technique was carried out separately. The control setup was also maintained. All these were incubated at 37^{o}C for 24 hours, and bacterial growth was observed. The above procedure was repeated to other bile concentrations of 0.5%, 1% and 1.5%.

pH tolerance test: 1.3g Nutrient broth was dissolved in 100 ml distilled water. Then the pH value was adjusted to range from 2 to 8. 10ml of nutrient broth was poured into each test tube and plugged with cotton wool. After that, those tubes were autoclaved at 121^{o}C and 15 lbs/inch^2 pressure for 15 minutes. The concentration of different pure culture isolates was standardized by using the Mc Farland standard (0.5M). 1ml of inocula was transferred into the sterile nutrient broth separately. Then the above tubes were

incubated at 37^{o}C for 24 hours. After that, test tubes were observed for bacterial growth. One tube was kept as control. The positive result for the growth was indicated by the turbidity of the medium.

Motility test: The motility test was carried out by using the hanging drop method. Based on the morphological, biochemical and physiological characteristics, Lactobacilli bacterial isolates were identified. (Prabhurajeshwar et al., 2017, Sultana et al., 2016 and Somnath et al., 2017).

2.4. Study the antagonistic effect

2.4.1. Among the lactic acid bacterial isolates (Standard agar well method)

3.8g Muller-Hinton agar was dissolved in100ml distilled water, and then above medium was autoclaved at 121^{o}C and 15lbs/inch^{2} pressure for 15 minutes. After sterilization medium was transferred into sterile Petri dishes aseptically and allowed to settle.

0.1ml of the particular pure isolate was inoculated on the medium and spread by using a sterile spreader. Wells were made on the medium by using a sterile cork borer. 0.1 ml of other isolates were standardized on Mc Farland (0.5M) and then transferred into the well under aseptic conditions. Standard streptomycin is used. Plates were incubated at 37^{o}C for 24 hours, and the diameter of the clear zone was measured. The above procedure was repeated on the other nine bacterial isolates.

2.4.2 Between the lactic acid bacterial isolates and with identified bacterial cultures

The following bacterial cultures were used for this study, such as *Bacillus sp. Staphylococcus sp. Pseudomonas aeruginosa,Proteus sp.*and *Escherichia coli*. The standard agar well method was used to study the antagonistic effect of lactic acid bacterial isolates on some non-lactic acid bacteria.

2.5. Antibiotic sensitivity test

3.8g Muller-Hinton agar was dissolved in 100 ml distilled water. After that, this medium was autoclaved at 121^{o}C and 15 lbs/inch^{2} pressure for 15 minutes. Then above media was poured into sterile Petri dishes under aseptic conditions and allowed to settle. 0.1ml of each pure isolate was transferred in the centre of the Muller- Hinton agar plate with a sterile pipette and spread on the surface of the medium by using a sterile spreader. Then selected antibiotic discs (bacitracin 10, ampicillin 10mcg, gentamicin 10mcg and streptomycin 10mcg) were transferred on the medium. Plates were incubated at 37^{o}C for 24 hours, and the diameter of the clear zone was measured.

2.6. Determination of the lactic acid content by fermentation method

1% Bacteriological peptone, 0.5% NaCl and 1% Lactose without 0.1% phenol red were dissolved with distilled water, then pH was adjusted to 7.2 by using HCl and NaOH. This was dispended into a conical flask plugged with cotton wool. This was autoclaved at 121^{o}C, and 151 lbs/inch^{2} pressure for 15 minutes.

A loop full of each isolated culture was transferred into the medium separately. A control was also maintained. Then tubes were incubated at 37^{o}Cfor 24 hours. Then incubated sample was titrated against 0.1M NaOH with the addition of 2-3 drops of phenolphthalein indicator until the colour of the sample

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turned to light pink. The percentage of purity of lactic acid is calculated using the given formula (Jatindra et al., 2015)

$$R=\frac{V(titr) \times C(titr) \times Mw \times F \times 100}{1000 \times W(samp)}$$

Where,

R - % of Lactic acid

V (titr) - Total volume of titrant needed to reach the endpoint in ml

C (titr) - Concentration of titrant

M.W-Molecular weight of lactic acid = 90.08

F -Dilution factor

W (Sam) - Sample amount in either gram or ml.

3. Results and Discussion

3.1 Preparation of curd by the traditional method

Table 1: Selected curd by the addition of lime.

Type of milk	Character	Volume of lime (in ml)							
		1	2	3	4	5	7	9	10
Cow milk	Whey content (in ml)	5	3	6	5	8	10	11	14
	Colour	White	White	White	White	White	White	White	White
	Texture	Watery Nature	Solid nature	Watery nature	Watery nature	Watery nature	Watery nature	Watery nature	Watery nature
	Whey content (in ml)	4	3	0	4	8	10	9	11
	Colour	White	White	White	White	Yellow	Yellow	Yellow	Yellow

Goat milk	Texture	Solid nature	Solid nature	Solid nature	Watery nature	Watery nature	Watery nature	Watery nature	Watery nature

Table 2: Selected curd by the addition of curd.

Types of milk	Character	Amount of curd in g							
		2	3	4	5	7	8	9	10
Cow milk	Whey content	3	2	1	8	9	10	12	11
	Colour	White	White	White	White	White	White	White	White
	Texture	Solid Nature	Solid Nature	Solid Nature	Watery nature	Watery nature	Watery nature	Watery nature	Watery nature
Goat milk	Whey content	4	4	2	5	6	8	8	10
	Colour	White	White	White	Yellow	Yellow	Yellow	Yellow	Yellow
	Texture	Solid nature	Solid nature	Solid nature	Watery nature	Watery nature	Watery nature	Watery nature	Watery nature

The best quality curd of cow milk was selected from 2ml lime added and 4g starter culture added samples, while curd of goat milk was selected from 3ml lime added and 4g starter culture added samples. Although curd formation occurred in all test samples (In different amounts of lime and starter culture), the above-pointed samples were considered of good quality due to the appearance of proper texture, proper colour and decreased whey content.

3.2 Isolation of lactic acid bacteria from prepared curd

Ten different lactic acid bacterial isolates were obtained from selected curd and labelled as CL1, CL2, CL3, CC1, CC2, CC3, GL1, GL2, GC1 and GC2.

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Table 3: Different lactic acid bacterial isolates from selected curd

Isolates code	Source
CL	Cow milk lime added
CC	Cow milk inoculum added
GL	Goat milk lime added
GC	Goat milk inoculum added

3.3 Characterization of Lactic acid bacteria

Table 4: colony characteristics of Lactobacilli isolates.

No	Selected lactobacilli	Colony characters					Cell characters	Arrangement
		Shape	Edge	Colony form	Colour	Colony		
1	CL 1	Circular	Convex	Punctiform	Pale yellow	Entire	Cocci	Mostly in chain
2	CL 2	Spread colony	-	-	-	-	Long rods	Mostly in chain
3	CL 3	Circular	Convex	Circular	Pale yellow	Entire	Long rods	Mostly in chains
4	CC 1	Circular	Convex	Irregular	Pale yellow	Entire	Long rods	Mostly in chains
5	CC 2	Circular	Convex	Circular	Pale yellow	Entire	Small rods	Mostly in single
6	CC 3	Circular	Convex	Circular	Pale yellow	Entire	Long rods	Mostly in single

7	GL 1	Circular	Convex	Irregular	Pale yellow	Entire	Small rods	Mostly in single
8	GL 2	Irregular	Convex	Irregular,	Pale yellow	Entire	Small rods	Mostly in single
9	GC 1	Circular	Convex	Circular	Pale yellow	Entire	Coccus	Mostly in tetrads
10	GC 2	Circular	Convex	Circular	Pale yellow	Entire	Small rod	Mostly in single

3.3.2 Biochemical characterization of bacterial isolates

Catalase test - Selected all isolates are catalase-negative.

3.3.2.2 Carbohydrate fermentation test

Table 5: Fermentation of different Carbohydrates by Lactic acid bacterial isolates.

No	Sugars	Isolates									
		CL 1	CL 2	CL 3	CC 1	CC 2	CC 3	GL 1	GL 2	GC 1	GC 2
1	Lactose	-	-	-	-	+	+	+	+	+	+
2	Sucrose	+	-	-	-	+	+	+	+	+	+
3	Glucose	+	+	+	+	+	+	+	+	+	+
4	Fructose	+	-	-	-	+	+	+	+	+	+
5	Galactose	+	-	-	-	+	+	+	+	+	+
6	Maltose	+	-	-	-	+	+	+	+	-	+
7	Mannitol	+	-	-	-	+	+	+	+	+	+

\+ Positive result - Negative result

Hydrolysis of starch and Gelatin:

(+) ve result: - indicate the Acid and Gas production

(-) ve result: - indicate the absence of Acid and Gas production

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Nitrate reduction test: All bacterial isolates showed Positive results for this test.

Positive result - Indicate the no gas production in all lactic acid bacterial isolates.

Indole test: Selected all isolates showed a Negative results.

3.3.3 Physiological characterization of bacterial isolates

NaCl tolerance test:

Table 7: Effect of different NaCl tolerance on Lactic acid bacterial isolates.

+++ High bacterial growth ++ Medium growth + Lower growth - No or poor growth

No	NaCl concentration	Isolates									
		CL 1	CL 2	CL 3	CC 1	CC 2	CC 3	GL 1	GL 2	GC 1	GC 2
1	3%	+	+++	+	+	++	++	++	+	++	+
2	4%	+++	++	++	-	++	++	++	-	++	++
3	5%	+++	++	++	-	++	++	++	-	++	++
4	6%	+++	++	+	-	++	++	+	-	++	+
5	7%	+++	++	+	-	+	++	+	-	++	+

pH tolerance test:

Table 8: Effect of different pH on Lactic acid bacterial isolates.

No	pH	Isolates									
		CL 1	CL 2	CL 3	CC 1	CC 2	CC 3	GL 1	GL 2	GC 1	GC 2
1	2	-	+	-	-	+	+	+	-	-	+
2	3	+	+	+	+	+	+	+	+	+	+
3	4	+	+	+	+	+	+	+	+	+	+
4	5	+	+	+	+	+	+	+	+	+	+
5	6	+	+	+	+	+	+	+	+	+	+
6	7	+	+	+	+	+	+	+	+	+	+
7	8	_	+	+	+	+	+	+	+	_	_

\+ Growth is observed - No growth

Bile tolerance test:

Table 9: Effect of different bile salt on Lactic acid bacterial isolates.

U.C -uncountable growth S.G – Spread growth

No	% of Bile	Isolates(Number of colonies /0.1ml									
		CL 1	CL 2	CL 3	CC 1	CC 2	CC 3	GL 1	GL 2	GC1	GC 2
1	0.3%	U.C	-	-	-	S.G	S.G	3	-	-	-
2	0.5%	181	-	-	-	S.G	S.G	33	28	66	31
3	1%	100	150	-	5	S.G	S.G	-	-	20	15
4	1.5%	25	3o	-	-	300	-	-	-	-	-

Motility test: Selected all isolates are non-motile.

Identified isolates of Lactobacilli:

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Table 10**:** Identified Lactic acid bacterial isolates.

NO	Isolates	Identified the isolates
1	CL 1	*Leuconostoc sp.*
2	CL 2	*Lactobacillus sp.*
3	CL 3	*Lactobacillus sp.*
4	CC 1	*Lactobacillus sp.*
5	CC 2	*Lactobacillus sp.*
6	CC 3	*Lactobacillus sp.*
7	GL 1	*Lactobacillus sp.*
8	GL 2	*Lactobacillus sp.*
9	GC 1	*Pediococcus sp.*
10	GC 2	*Lactobacillus sp.*

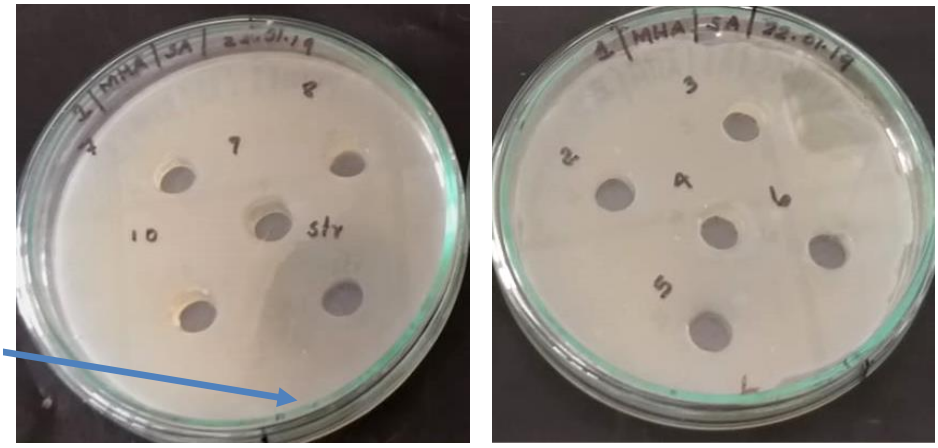
Isolates code	Source
CL	Cow milk lime added
CC	Cow milk inoculum added
GL	Goat milk lime added
GC	Goat milk inoculum added

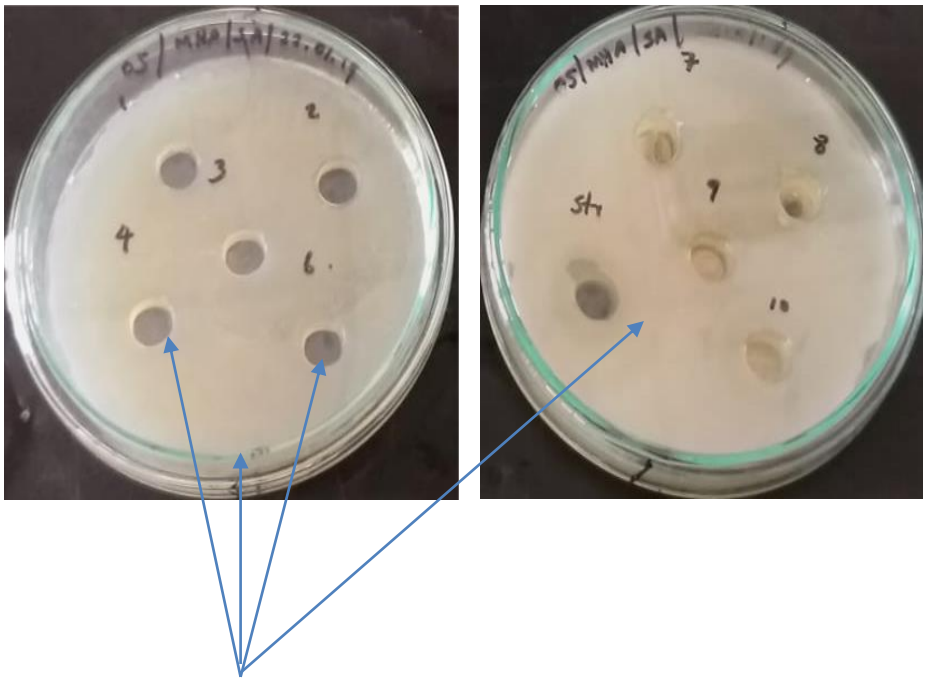
3.4 Antagonistic effect of bacterial isolates

3.4.1. Among Lactic acid bacterial isolates (standard agar well method)

There were no clear zones observed around any of the isolates.

Clear zone obtained for standard Streptomycin





No clear zone

Figure 1. Antagonistic effect among different Lactic acid Bacteria.

Antibacterial activity was observed by the formation of a clear zone. Here no clear zones were observed; therefore, antibacterial activity was not exhibited by all identified Lactobacilli. Standard streptomycin inhibited the growth of all Lactobacilli. Clear zone formation depends on the concentration of the bacterial inocula, type of bacteria, type of medium and incubation conditions.

3.4.2. Between the Lactic acid bacterial isolates with some identified bacterial cultures

Table 11: Effect of Lactic acid bacterial isolates on the growth of other bacteria.

No	Isolates codes	Average Zone of inhibition in mm				
		Bacillus sp.	*Pseudomonas aeruginosa*	*Proteus sp.*	*E coli*	*Staphylococcus sp.*

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1	CL 1	13.93	-	14.38	-	17.14
2	CL 2	12.05	13.54	14.17	13.54	17.14
3	CL 3	14.34	-	-	-	-
4	CC 1	-	13.70	-	13.70	-
5	CC 2	15.93	19.32	27.31	19.32	15.21
6	CC 3	13.85	-	17.35	-	12.79
7	GL 1	12.71	-	15.8	-	-
8	GL 2	13.40	-	-	-	15.22
9	GC 1	13. 59	-	-	-	-
10	GC 2	14.76	-	-	-	-

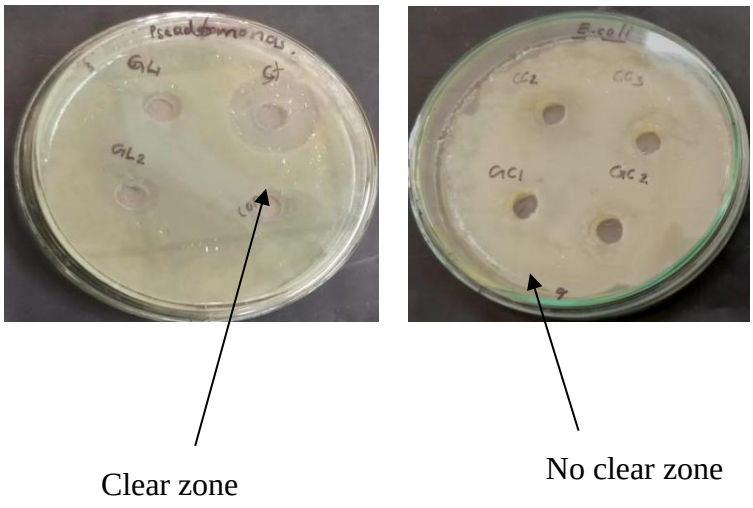


Figure 2. Effect of Lactic acid bacterial isolates on the growth of other bacteria.

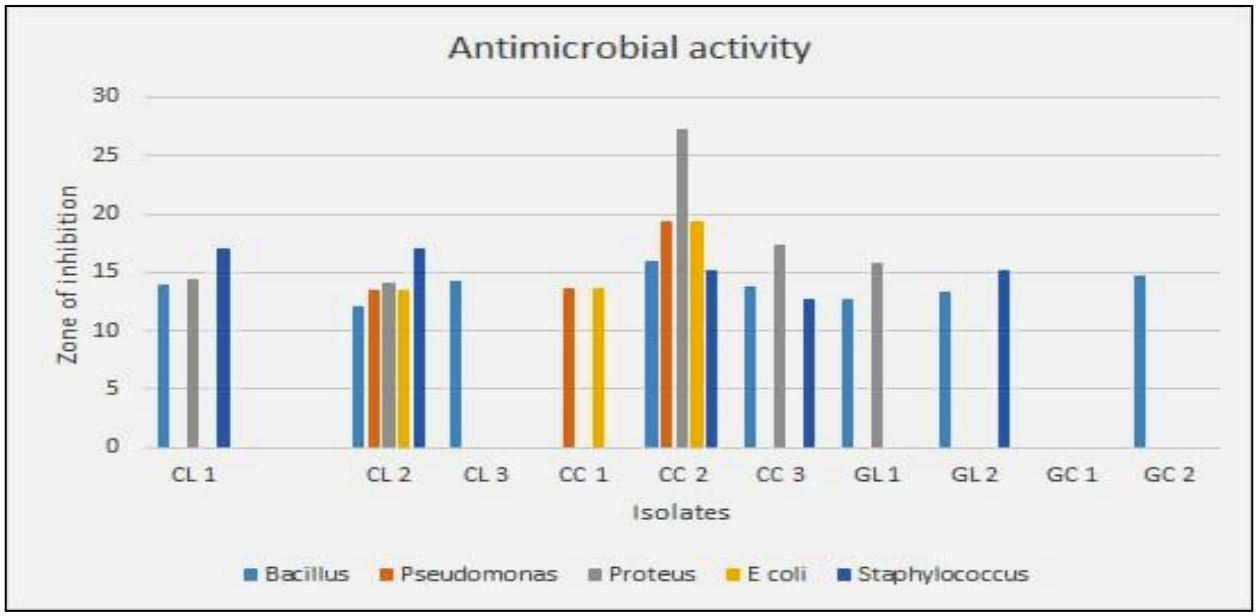
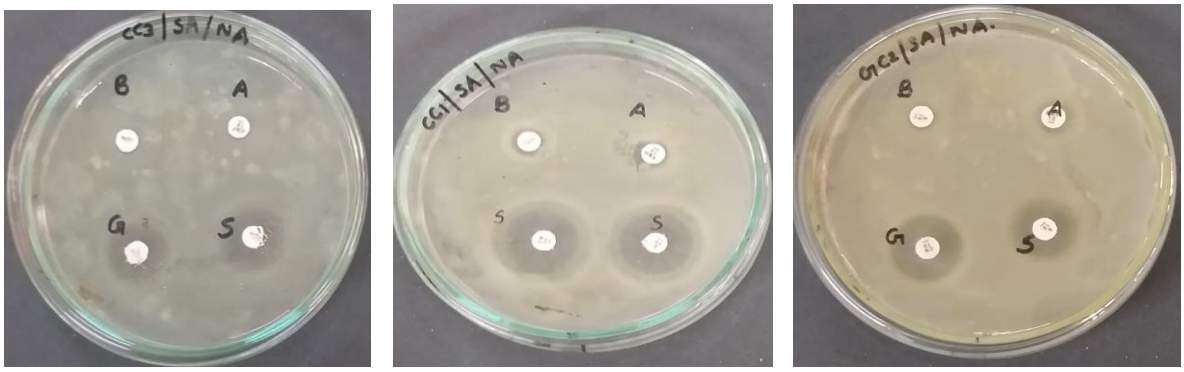


Figure 3. Antibacterial effect of Lactobacilli isolates against selected bacterial species.

In this study, lactic acid bacterial isolates inhibited the growth of selected bacterial species. The degree of inhibition varied among the Lactobacilli as well as among the selected bacteria. The growth of *Bacillus sp.* was predominantly inhibited by Lactic acid bacterial isolates (90%); likewise, *Pseudomonas aeruginosa* (30%), *Proteus sp.* and *Staphylococcus aureus* (50 %), and *E.coli* (30%), *Lactobacillus sp*. (CL2) predominantly inhibited all tested bacteria.(100%). The degree of growth of inhibition by *Lactobacillus sp.* (CC2) was high on *Bacillus sp.*, *Pseudomonas aeruginosa*, *Proteus sp.* and *E.coli* than in the other isolates. However, comparatively the degree of growth inhibition by *Leuconostoc sp*. (CL1) and *Lactobacillus sp.* (CL2) was high on *Staphylococcus aureus* than in other isolates.

3.4 Antibiotic sensitivity test



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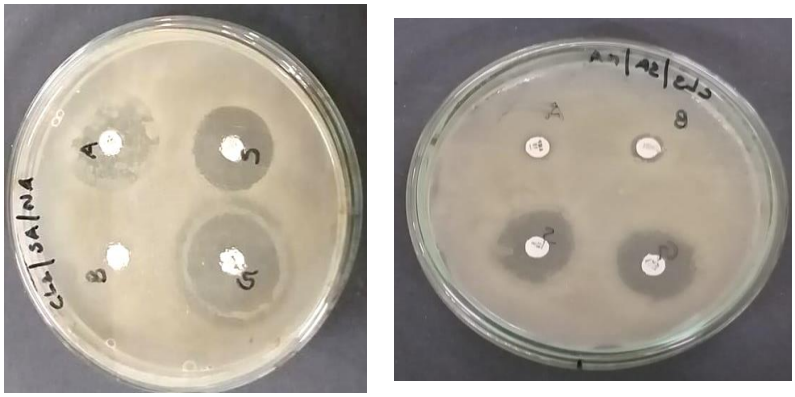

Figure 4. Antibiotic sensitive test. A- Ampicillin, B- Bacitracin, G- Gentamycin and S- Streptomycin.

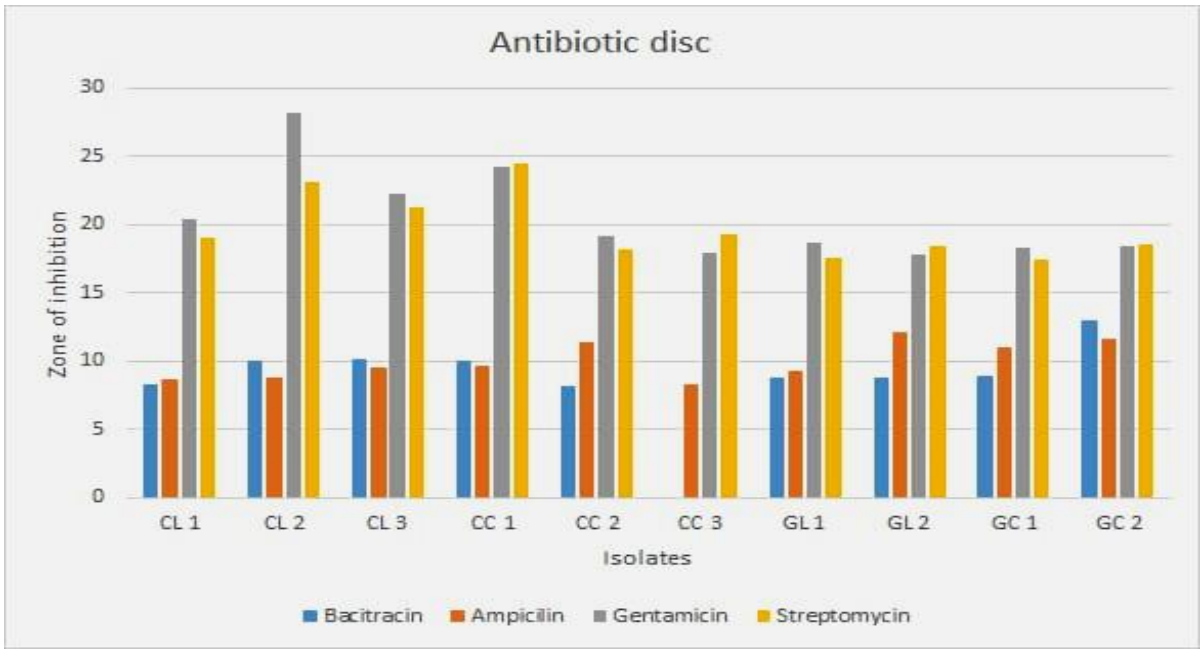

Figure 5. Antibiotic sensitivity test for Lactobacilli isolates.

Antibiotic susceptibility of Lactic acid bacteria is one of the crucial criteria from the safety point of view of potential probiotics. Resistance of the probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections.

According to this study, the various species of Lactobacilli were sensitive to different antibiotics. Based on that, all of the bacterial isolates were sensitive to all four antibiotics, while one of the *Lactobacillus sp.* (CC3) was resistant to bacitracin only. *Lactobacillus sp.* (CL2) is predominantly sensitive (28.19mm) to gentamycin, while *Lactobacillus sp.* (CC1) is predominantly sensitive(24.5mm) to streptomycin. *Lactobacillus sp*. (GC2) is predominantly sensitive (13.05mm) to bacitracin and

Lactobacillus sp. (GL2) is sensitive (12.16mm) to ampicillin. *Lactobacillus sp*. (CC2), *Lactobacillus sp*.(CC3), *Lactobacillus sp*.(GL2) and *Pediococcus sp*.(GC1) were less sensitive to bacitracin (8.2mm), ampicillin (8.31), gentamycin (17.85mm) and streptomycin(17.44) respectively.

3.6. Determination of the lactic acid content by fermentation method

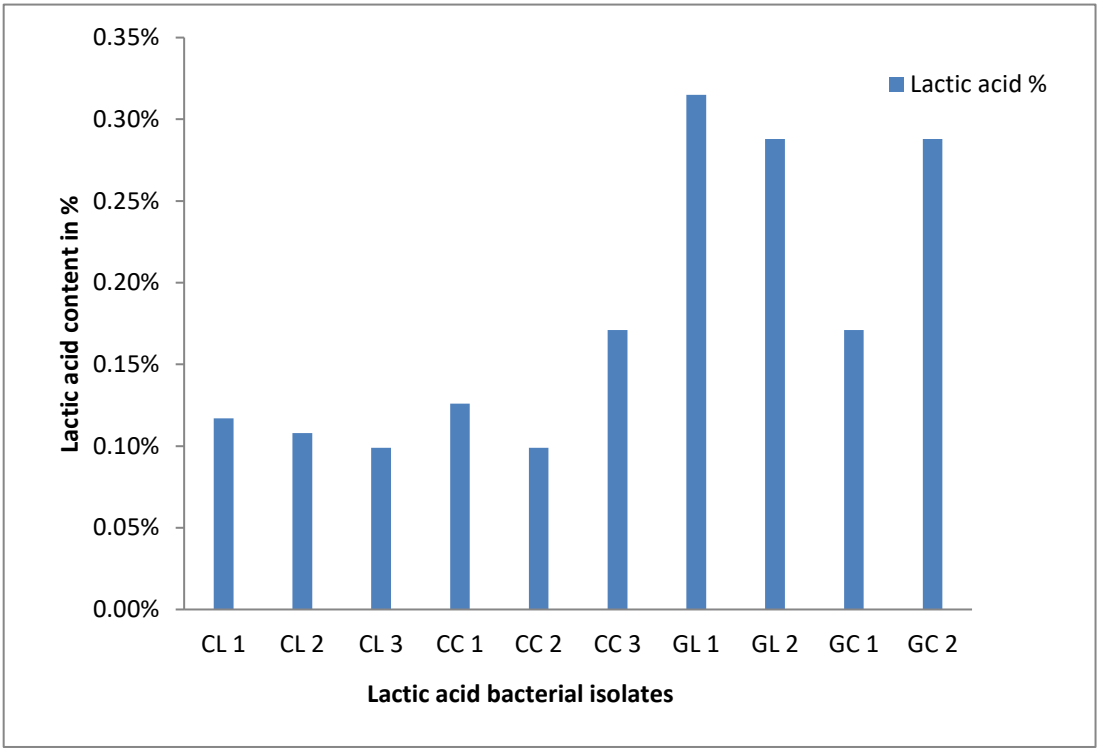

Figure 5. The lactic acid content of different Lactic acid bacteria.

All of the Lactic acid bacterial isolates produced a considerable amount of Lactic acid. Under the controlled conditions, the *Lactobacillus sp.* (GL1) produced a significantly high level of lactic acid. (0. 315%), whereas *Lactobacillus sp*. (CL3) produced less amount of lactic acid. (0.099%)

Lactic acid fermentation is a metabolic process by which initially glucose and other six-carbon sugars (sucrose or lactose) are converted into cellular energy and the metabolite lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells.

Lactic acid bacteria can be conveniently divided into two groups: Homofermentative and heterofermentative. Homofermentative types ``produce lactic acid as the major or sole product of glucose fermentation, whereas the heterofermentative types produce equal molar amounts of lactic acid, carbon dioxide and ethanol. The genera *Pediococcus*, *Streptococcus*, *Lactococcus* and *Vangococcus*are all exclusively homofermentative, whereas the important *Lactobacillus* genus comprises homofermentative and heterofermentative species.

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4. Conclusion

Ten Lactobacilli isolates from traditionally prepared curd were identified, and those belonging to three different genera as *Lactobacillus sp.*, *Pediococcus sp.* and *Leuconostoc sp.* Growth of all Lactobacilli was observed under acidic and neutral pH. Lactic acid bacteria exhibited different tolerant levels to bile salt. No antagonistic effect was observed among the Lactobacilli isolates. 80% of lactobacilli isolates had antibacterial activity on selected bacterial species. Growth of the Lactobacilli isolates was inhibited by tested antibiotics such as bacitracin, gentamycin, ampicillin and streptomycin. Lactic acid production varied among the Lactobacilli isolates.

Further studies could be carried out to optimize the lactic acid production in different Lactobacilli and also their consortia.

References

Hutt, P., Shchepetova, J., Loivukene, K., Kullisaar, T. andMikelsaar, M. (2006) Antagonistic activity of probiotic Lactobacilli andbifidobacteria against entero- and uropathogens,Journal of Applied Microbiology. 100: 1324–1332.

Katawal, SB. and Subba, D. (2008). A Survey study on Technology of Sel-roti: A traditional food of Nepal Food Science and Technology Nepal. 4: 23-30.

M. Y. Ali, M. A. Islam, M. J. Alam and M. N. Islam, 2002. Quality of Yogurt (Dahi) Made in Laboratory and Available in the Market of Mymensingh Town in Bangladesh. Pakistan Journal of Biological Sciences, 5: 343-345.

Leroy, F. and De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry, Trend in Food Science and Technology, 15(2): 67-78.

Mohan,K.R.Shankar,P.A. and Laxminarayana,H 1985.Quality of Dahi (curd) manufactured from buffalo milk. Dairy Science Abstracts.48: 7390.

Patel, A., Prajapati, J.B., Holst, O. and Ljungh, A. (2014). Determining probiotic potential of exo polysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products, Food Biosciences. 5:27-33

Prabhurajeshwar,C., and Chandrakanth,R.K. (2017). Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances, biomedical journal.40(5):270-283.

Somnath., Pramanik,A., Aditya,K.R., Suchismita,P., Priyanka,P. and Sourav,J. (2017). Isolation and characterization of *Lactobacillus sp*. from curd and its pharmacological application in probiotic chocolate, The Journal of Phytopharmacology.6(6): 335-339.

Sultana,J.M., Refaya,R., Shajidur Rahman, Md. And Kohinur, B. (2016). Isolation and Biomedical characterization of Lactobacillus species from Yogurt and Cheese samples in Dhaka Metropolitan Area, Bangaladesh Pharmaceutical Journal. 20(1): 27-33.

Tamime, AY. Robinson, RK. (1999). Yoghurt: Science and Technology. International journal of Diary Technology. 52(4): 148-148