

EFFECT OF STARTER CULTURE ON DEVELOPMENT OF CURD (DAHI) AND THEIR ANTAGONISTIC PROPERTY AGAINST SOME ENTERIC PATHOGEN

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ABSTRACT

Background: Curd is an important fermented food which is generally consumed by the community people in large scale and becomes a functional upon incorporating probiotics-live microorganisms due to a great variety of positive health effects.

Methods: In this study, pure cultures of *Lactobacillus casei* (MTCC1408), *L fermentum* (MTCC 903), *L rhamnosus* (MTCC1462), *L ingluviei* ADK10 (GenBank Accession No-JQ395039), *Enterococcus faecium* ADK18 (KF032592.1), *E durans* ADK14 (KF032593.1) and *Streptococcus thermophilus* (MTCC 1938) were inoculated to pasteurized Amul Milk separately to produce curd. Different physiochemical and antimicrobial characteristic of curd against some pathogen were analyzed to assay the quality of curd.

Results: The results of the study revealed that the moisture content of the curd samples ranged between 86.36%- 88.71% and the pH values of the samples ranged between 3.96-5.43, which were reasonably suitable for curd processing industries in tropical countries. Titratable acidity (0.81-1.71), protein (range 1.4g-9.6g) and fat (2.2g-6.8 g) content also varied with respect to different lactic acid bacterial strains. All seven lactic acid bacteria (LAB) strains showed their antagonistic activity (zone of inhibition 5-10 mm) against four enteropathogenic bacterial strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 15380, *Streptococcus mutans* ATCC 25175, *Bacillus subtilis* ATCC 6633). *L ingluviei* ADK10 and *L. rhamnosus* strains showed wide inhibitory spectrum against all the tested strains.

Conclusions: It can be conclude that the physiochemical parameters were varies according to their starter culture with antimicrobial activity against some entero-pathogens and has been fruitful starter culture for the preparation of curd having better health promoting effects.

Key words: Curd, Physiochemical evaluation, antagonistic activity.

INTRODUCTION

Curd is well known dairy product obtained by Lactic Acid fermentation of milk. It is generally consumed in its original form as an accompanied to the meal or it may be turned into raita by mixing it with grated cucumber, diced boiled potato, fried bits of gram flour batter, or pulsed based vadas. Curd or Dahi may be consumed as a sweet or savory lassi drink or as a dessert containing sugar and fresh diced banana, orange slices, mango bits and other seasonal fruits. In India system of medicine (Ayurveda), curd has been strongly recommended for curing ailments like dyspepsia, dysentery and other gastrointestinal disorders. This product is

also believed to improve appetite and vitality. Some of the beneficial effects of curd are attributed to the antibacterial components formed during the fermentation and the low pH that prevents the growth of putrefactive and other undesirable organisms including potential pathogens and possesses an increased digestibility (1). Further it balanced the fecal enzymes and intestinal micro flora, prevention of cancer, treatment of traveling diarrhoea, antibiotic therapy, and control of ulcer and reduction of serum cholesterol (2).

In recent years, it has been reported that lactic culture increases the vitamins and free amino acid contents of curd. Certain microbes are capable of colonizing

the lower intestine, improving gastrointestinal health and consequently enhancing immune function (3). The addition of specific lactic acid bacteria to milk used in the production of curd enhances the digestibility and nutrient value of milk. It is also known as probiotic or functional food as it possesses live lactic acid bacteria. Lactic acid is generated by bacteria as a result of the breakdown of carbohydrates. This process effectively lowers the pH of the food product to the point where the proliferation of pathogenic microorganisms reduced due to certain antibacterial substances were produced by starter cultures, resulting in the inactivation of undesirable microorganisms in cultured dairy products.

In the present study we were selected some pure cultures of lactic acid bacteria (*L. casei*, *L. fermentum*, *L. rhamnosus*, *L. ingluviei* ADK10, *E. faecium* ADK18, *E. durans* ADK14 and *S. thermophilus*) and were inoculated to pasteurized Amul milk separately to produce curd. All the said culture were nonpathogenic and experiment was done on rat model(4,5,6,7). Different physiochemical and antimicrobial characteristic of curd against some pathogen were analyzed to assay the quality of curd.

MATERIALS AND METHODS

Selection of Strain:

Bacterial strain of *Lactobacillus casei* MTCC1408, *L. fermentum* MTCC 903, *L. rhamnosus* MTCC1462, *Streptococcus thermophilus* MTCC 1938 were obtained from microbial type culture collection (MTCC) and the pure culture of *L. ingluviei* ADK10(GenBank Accession No-JQ395039), *Enterococcus faecium* ADK18(KF032592.1), *E. durans* ADK14 (KF032593.1) were obtained from the Department of Microbiology, Raja N.L. Khan Women's College. These cultures were selected for the development of curd in the present study. For showed the comparative cell and colony character of all selected strain (phenotypic characteristics i.e. colony characteristics, gram staining, cell shape and catalase production) were plated onto de Man-Rogosa-Sharpe (MRS) agar [composition (w/v) 1.0% peptone, 0.8%

meat extract, 0.4% yeast extract, 2.0% glucose, 0.5% sodium acetate trihydrate, 0.1% polysorbate 80 (also known as Tween 80), 0.2% dipotassium hydrogen phosphate, 0.2% triammonium citrate, 0.02% magnesium sulfate heptahydrate and 0.005% manganese sulphate tetrahydrate, pH 6.5] and incubated at 37°C under anaerobic condition for 24-48 h.

Preparation of curd:

Seven conical flask, each containing 100 ml of Standardized amul milk was heated to 85°C for 30 minutes, cooled to 40°C. The milk samples were inoculated individually at 1% level with a specific species of *Lactobacillus casei*, *L. fermentum*, *L. rhamnosus*, *L. ingluviei* ADK10, *Enterococcus faecium* ADK18, *E. durans* ADK14 and *Streptococcus thermophilus* under the laminar air flow chamber to avoid contamination. All samples were incubated at 37°C for 48 hours for activation of culture to set the curd (8).

Sensory Evaluation:

Body, colour and flavor was evaluated by the method describe by Stephanie et.al (9).

Moisture content determination:

The moisture content of the curd products was determined according to the Association of Official Analytical Chemists method (AOAC, 1995). Each curd product (10 g) was placed in an oven at 105°C for 3 h. Reading was taken at a constant weight. The moisture content was then expressed as the percentage (%) of the dry weight of sample (10).

$$M \% = \frac{W1 - W2}{W} \times 100$$

Where, M = Moisture, W1 = Weight of plate & curd, W2 = Weight of plate & curd after drying.

Total solids:

The weight of the residue obtained from moisture content analysis was expressed as percentage total solids using the formula below (11).

Total solid (%) = weight of dish + Dry curd / weight of sample × 100

Physiochemical test:

All the curds were analyzed for body, color, flavour and texture, chemical quality- pH, Fat%, Protein%, carbohydrate% and titratable acidity% of curd. pH was measured by pH meter at different time interval, titratable acidity was measured with NAOH and phenolphthalin according to AOAC procedure (12) fat by Gerber’s Method, Protein was determined by the Kjeldahl method as per International Dairy Federation (IDF) (13), carbohydrate by Nelson-Somogi’s method and others vitamins and mineral contents measured as described by Aneja et.al. (14).

Detection of antimicrobial activity:

After incubation, cell free solutions of bacterial cultures were obtained by centrifugation (10 min × 15000g at 4°C) followed by filtration of the supernatant by 0.22 µm cellulose acetate filter. Supernatant of curds and pure curd

samples was taken for the antimicrobial test. Overnight broth culture of target strains (*Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus mutans*, *Bacillus subtilis*) were inoculated (0.1 ml) on solid Mueller-Hinton agar medium by spreading. After 10 minutes of contact, the plates were dried for 20 minutes. Six wells were made and filled with 100 µl of previously prepared cell free solutions. Target strain inoculated plate with un-inoculated MRS broth served as control. Plates were incubated at 37°C for 24 hours and diameter of inhibition zones were measured with calipers. The antimicrobial tests were done in duplicate and the mean values were recorded (10,15,16).

RESULTS AND DISCUSSION

Phenotypic Characteristics:

Cell and colony character of all selected strain were determined and tabulated (Table- 1). All the bacteria were gram positive, round in shape and catalase negative.

Table 1: Comparative cell and colony character of all lactic acid bacteria isolates and collected strains and primary screening profile.

Bacterial strain	Cell character		Colony character		Catalase activity
	Shape	Gram staining	Shape	Color	
Lactic acid bacteria isolates					
<i>L. ingluviei</i> ADK10	Plumped rod	Positive	Round	Grayish white	Negative
<i>E. durans</i> ADK14	Coccus	Positive	Round	Grayish white	Negative
<i>E. faecium</i> ADK18	Oval	Positive	Round	Grayish white	Negative
Strain from MTCC					
<i>Lactobacillus casei</i>	Oval	Positive	Round	Grayish white	Negative
<i>Lactobacillus fermentum</i>	Plumped rod	Positive	Round	Gray	Negative
<i>Lactobacillus Rhamnosus</i>	Plumped rod	Positive	Round	Milky white	Negative
<i>Streptococcus thermophilus</i>	Coccus	Positive	Round	Grayish white	Negative

Sensory Evaluation:

Body of the curd means the adhesion of particles of curd with each other and water released. LAB contributes to the aroma and flavour of fermented products.

They acidify the food, resulting in a tangy lactic acid taste, frequently exert proteolytic and lipolytic activities, and produce aromatic compounds. Wild strain starter cultures play an important role in flavour formation because they have a high

biosynthetic capacity and produce aromatic compounds. The addition of as adjunct cultures for curd manufacturing increases the level of free amino acids, peptides, and free fatty acids, leading to flavour intensity and accelerated cheese ripening. In our

study, it was showed that *L rhamnosus*, *L ingluviei* ADK10 was good result with compared to standard Amul dahi (Table 3).

Table 2: Growth temperatures and acid production moisture (%) & Total solid (%) by different starter culture for making curd with compared to Amul dahi*.

Starter Culture used for making curd	Growth temperature °C	Titration acidity (%)	Moisture (%)	Total solid (%)
<i>L. casei</i>	25-30	1.0-1.13	88.01±1.08 ^a	20.54 ± 1.31 ^a
<i>L. fermentum</i>		Small	87.36±1.38 ^b	23.39 ± 1.21 ^b
<i>L. rhamnosus</i>		1.41-2.0	87.56±0.68 ^b	22.59 ± 1.25 ^b
<i>L. ingluviei</i> ADK10		0.91-1.0	84.53±0.49 ^c	21.27 ± 1.46 ^b
<i>E. faecium</i> ADK18	35-40	0.83-1.0	87.59±0.84 ^b	18.47±1.74 ^c
<i>E. durans</i> ADK14		Small	88.33±1.13 ^a	16.72 ± 2.23 ^d
<i>S. thermophilus</i>		1.08-2.0	88.93±1.23 ^a	16.97±2.11 ^d
*Amul Dahi		0.88-1.0	85.37± 0.71^c	21.83±0.88^b

*Letters (a, b, c, d) in a column are significantly different at p<0.05

Table 3—Characteristic of curd prepared by selected starter culture

Starter culture	Body	color	flavour
<i>Lactobacillus casei</i>	Tough	White	Slight acidic
<i>Lactobacillus fermentum</i>	Firm	White	Fair
<i>Lactobacillus rhamnosus</i>	Tough	Cream	Good
<i>Lactobacillus ingluviei</i> ADK10	Tough	White	Very good
<i>Enterococcus faecium</i> ADK18	Tough	Cream	Fair
<i>Enterococcus durans</i> ADK14	Loose	Cream	Mild
<i>Streptococcus thermophilus</i>	Tough	White	Fair

Acidification Properties:

Rapid acidification is a main concern for development of starter cultures suitable for dahi production. It was revealed that the enzyme activity of starter bacteria used in bio-yoghurt production resulted in significant decrease in P^H as well as acidity during storage (17,18,19). Mean acidification data measured for the seven strains selected for the development of starter cultures are shown by species in Table 2. Strains within a species generally exhibited similar acidification activities in milk; this is seen by the low standard deviations of p^H. *L. fermentum* displayed the fastest acidification rate with a pH of

4.73±0.2 after 8 hours and the lowest pH of 3.96 after 48 hours (Fig. 1). Good acidification properties were also detected for *L. ingluviei* ADK10, *L. rhamnosus* and *L. casei* with pH 4.86, 4.64, 4.37 after 48 hours. Which were nearly similar to the standard Amul milk. Strains (curd) of the other species did not decreased pH below 5.0 within 48 hours. The P^H values of the samples were reasonably justified - and suitable for curd marketed in tropical areas because of the expected effect of bad storage conditions such as high temperatures encountering in some zones in India which can affect the acidity of curd.

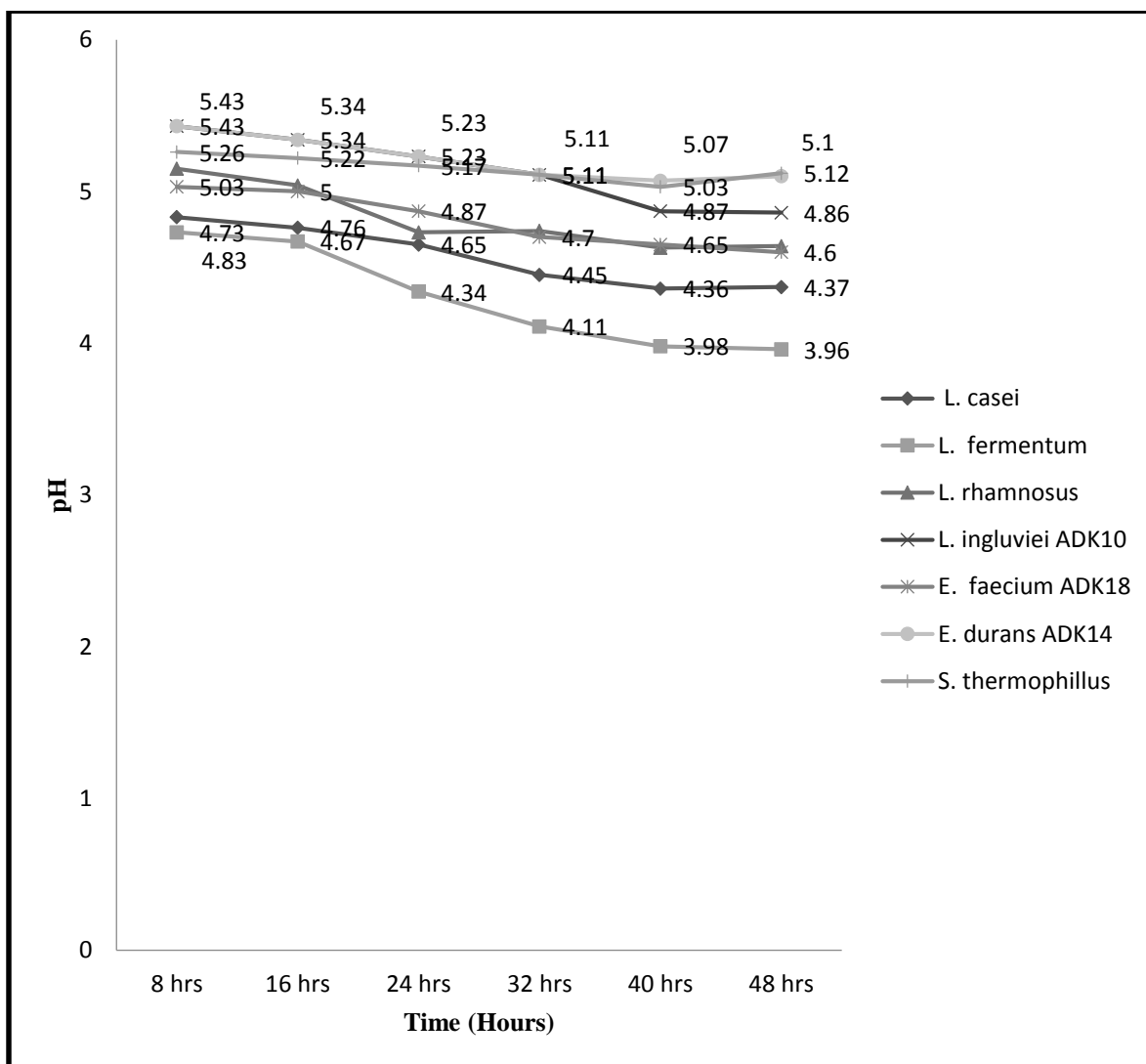


Fig. 1. Changes of pH at different Time Duration

Physico-Chemical Properties of the Experimental Curd:

Curd is one of the most important products in the family of fermented milks. The total quantity of milk converted into milk products, about 15% is used for Dahi making. Dahi is reported to have better nutritive value than milk. Though there was no increased in carbohydrate, protein and fat (Fig. 2,3,4) content of milk during fermentation (2). The chemical composition of Amul milk used for the production of Dahi fell within the following averages: titratable acidity 0.21, carbohydrate 35.9 %, fat 9 % and protein 6.23%. The changes of some physico-chemical properties of the

seven curd samples are presented table 2 & 4. Result showed that changes of vitamins and minerals of milk during curd formation were varied according to bacterial strain used. But it was not changed significantly in *L. rhamnosus* & *L. ingluviei* ADK10 curd with respect Amul milk content (Table 4) (Per 100 ml of milk content: Calcium-144mg, Phosphorous- 92mg, Vitamin A-106 I.U, Thiamine- 53 ug, Riboflavin- 157 ug, Nicotinic acid- 94 ug, Biotin- 31 ug, Folic acid-151 ug, Vitamin B12- 0.16 ug, Ascorbic acid - 1.3 mg.) whereas mineral and vitamin content decreased significantly compared with that of milk content.

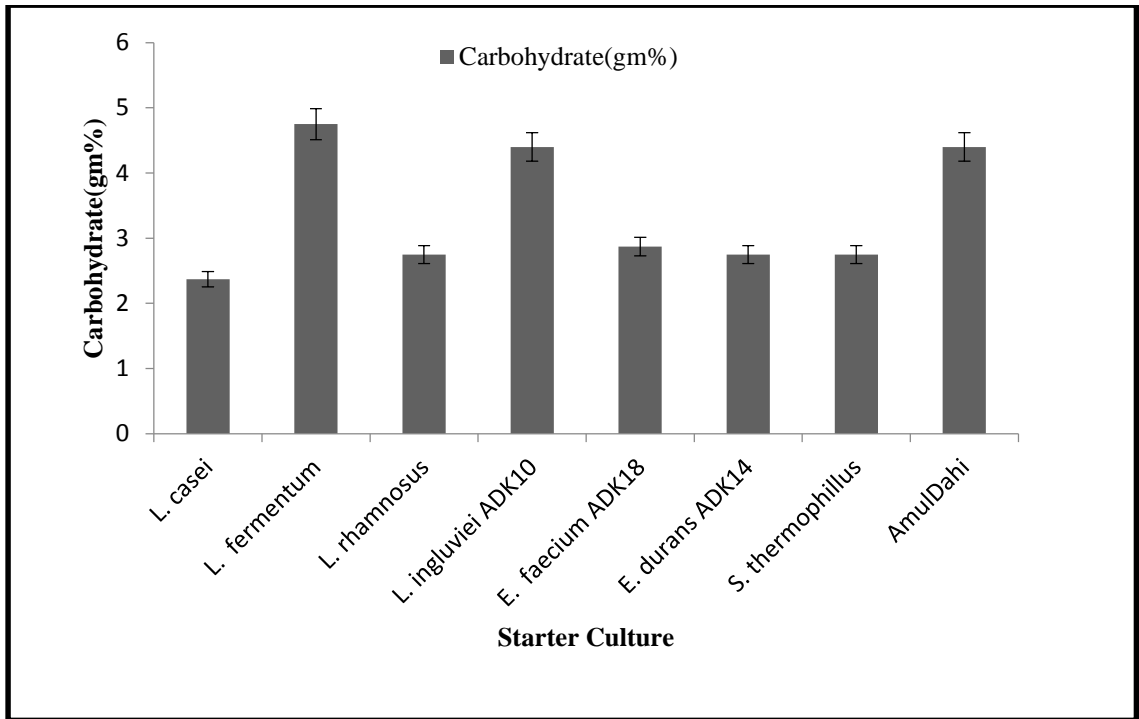


Fig. 2. Carbohydrate (gm%) of different type of curd sample.

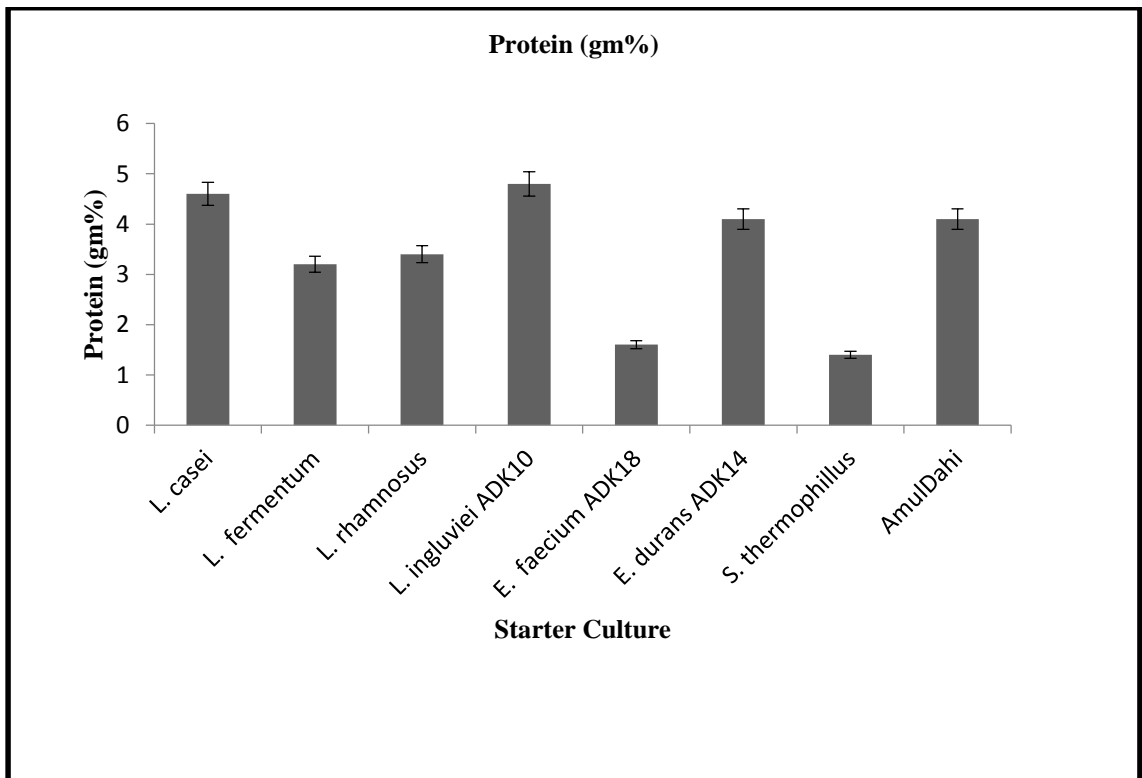


Fig. 3. Protein (gm%) of different type of curd sample.

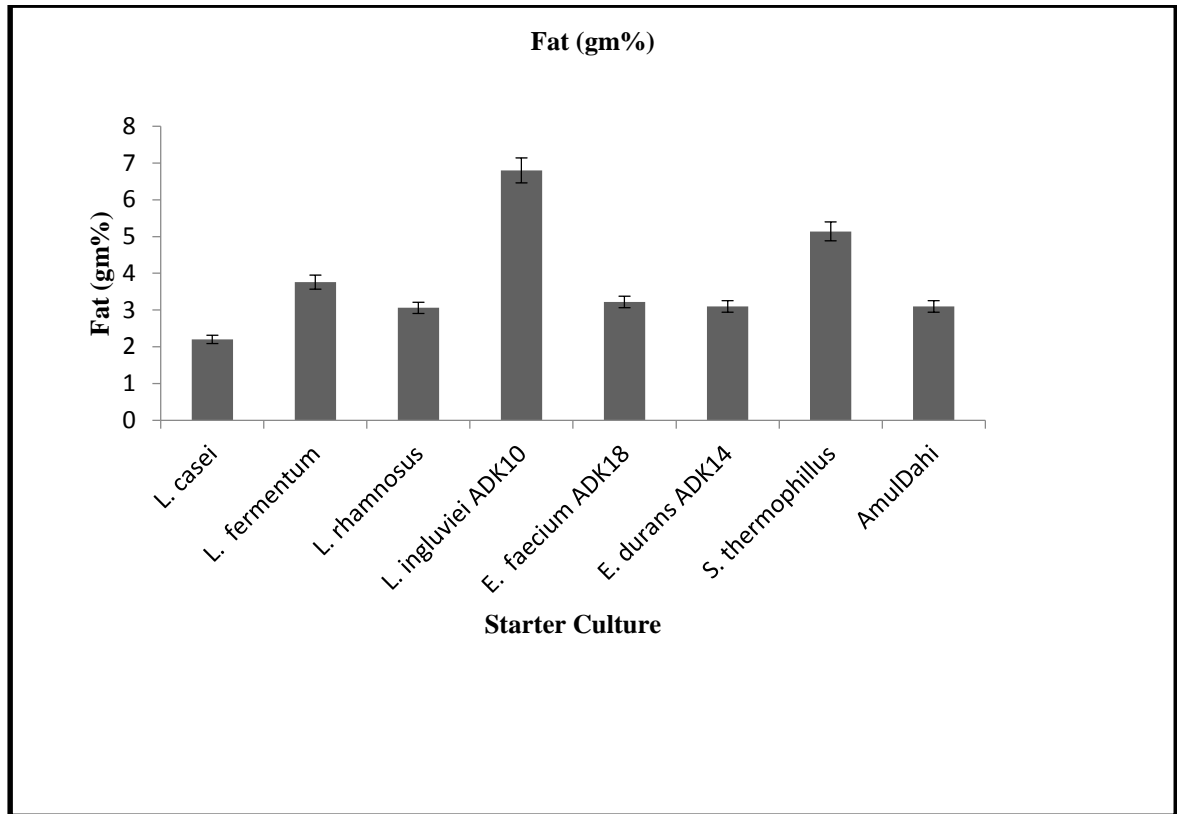


Fig. 4. Fat (gm%) of different type of curd sample.

Table 4: Chemical Composition of Amul Milk & Dahi

Constituent	*Milk	<i>L. casei</i>	<i>L. fermentum</i>	<i>L. rhamnosus</i>	<i>L. ingluviei</i> ADK10	<i>E. faecium</i> ADK18	<i>E. durans</i> ADK14	<i>S. thermophilus</i>
Calcium (mg)	144±0.57 ^a	143±0.88 ^a	142.33±0.88 ^b	142.66±0.66 ^b	143.33±0.66 ^a	142±0.86 ^b	143±1.0 ^a	142.6±0.66 ^b
Phosphorous (mg)	92±0.28 ^a	85±0.57 ^b	85.33±0.3 ^b	86.33±1.20 ^b	88.33±0.66 ^c	86.6±1.85 ^b	85±0.57 ^b	80.6±0.80 ^d
Vitamin A (I.U.)	105.6±0.80 ^a	85.3±0.88 ^b	81±0.57 ^c	90.6±1.4 ^a	90±1.15 ^d	80±0.57 ^c	83.6±0.30 ^a	79.6±0.80 ^c
Thiamine (ug)	53±1.15 ^a	41±0.57 ^b	38.6±0.88 ^c	45.6±0.80 ^d	46.5±0.50 ^d	35±1.52 ^a	41.6±1.20 ^b	34±0.57 ^a
Riboflavin (ug)	157.3±1.45 ^a	141±1.15 ^b	139±0.80 ^c	146±1.45 ^d	141±1.76 ^b	125±1.33 ^a	131.3±0.88 ^f	134±0.57 ^e
Nicotinic acid (ug)	93±0.57 ^a	80.3±1.45 ^b	78±0.57 ^b	83±0.57 ^c	83.3±0.88 ^c	82.3±1.45 ^c	79±1.0 ^b	75±1.14 ^d
Biotin (ug)	31±1.15 ^a	-	1.3±0.05 ^b	4.9±0.11 ^c	3.06±0.13 ^d	1.3±0.20 ^b	1.93±0.32 ^b	-
Folic acid (ug)	150±2.64 ^a	153±0.57 ^b	153.6±1.3 ^b	170±2.08 ^c	167±1.52 ^d	163.3±1.2 ^a	153.3±0.88 ^b	156.3±1.20 ^a
Vitamin B12(ug)	0.16±0.01	-	-	-	-	-	-	-
Ascorbic acid (mg)	1.3±0.08 ^a	0.7±0.11 ^b	0.73±0.12 ^b	1.2±0.17 ^c	1.1±0.1 ^c	1.06±0.03 ^c	0.53±0.08 ^d	0.76±0.17 ^b

*Values given per 100 gm of product

Data with different superscripts (a, b, c, d, e, f, g) in a specific row differ from each other significantly ($p < 0.05$).

Antimicrobial Activity:

Antimicrobial activity of the seven curd samples were detected against four enteropathogenic bacterial strains: *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus mutans*, *Bacillus subtilis*. Lactic acid bacteria are reported to produce some antimicrobial substances that are inhibitory for spoilage and pathogenic bacterial strains. Low molecular mass substances like lactic acid (also lower the medium pH), H₂O₂, CO₂, ethanol, diacetyl (also a flavouring agent) and high molecular mass compounds like bacteriocins are reported to be produced by LAB present in milk or fermented milk products (20,21). In the present study both the cell free solution (supernatant) and Pure curd of the seven

strains were tested to know if the antimicrobial metabolites were extracellular and released into the growth medium (15). All the bacteria showed inhibition against tested strains to varying degrees (Table 5). The inhibitory products are extracellular and diffusible. All curd sample were active against more than one tested strains (range from 5-10 mm). For example, we have used 7 LAB strains CFS and experimental Dahi inoculated with them as their Starter culture in respect to control. *L. rhamnosus* (MTCC1462), *L. ingluwiei* ADK10 two of them showed inhibition against both gram positive and gram negative pathogenic strain and most promising result as CFS and crude curd in respect to control (Fig. 5).

Table 5: Antimicrobial effect of curd produce by different starter culture

Curd Sample	Name of pathogens and spoliage bacteria							
	<i>S. mutan</i>		<i>B. subtilis</i>		<i>E.coli</i>		<i>K. pneumonia</i>	
	Super-natant	Curd	Super-natant	Curd	Super-natant	Curd	Super-natant	Curd
<i>L. casei</i>	+++	-	+	-	++	++	++	++
<i>L. fermentum</i>	-	+	-	+	-	-	-	-
<i>L. rhamnosus</i>	+++	+++	+	+++	+	+++	-	+
<i>L. ingluwiei</i> ADK10	++++	+++	++++	+++	+++	+++	+++	++
<i>E. faecium</i> ADK18	-	-	-	+	+	+	++	+
<i>E. durans</i> ADK14	-	++	-	+	-	+++	-	++
<i>S. thermophilus</i>	-	+	-	-	-	-	-	-
<i>Amul dahi</i>	+++	-	++	+	++	+	++++	++

*Inhibition Zone ≥ 15 mm (++++), Zone ≥ 10 mm(+++), Zone ≥ 5 mm(++) , Zones ≤ 5 mm (+), Negative (-) .

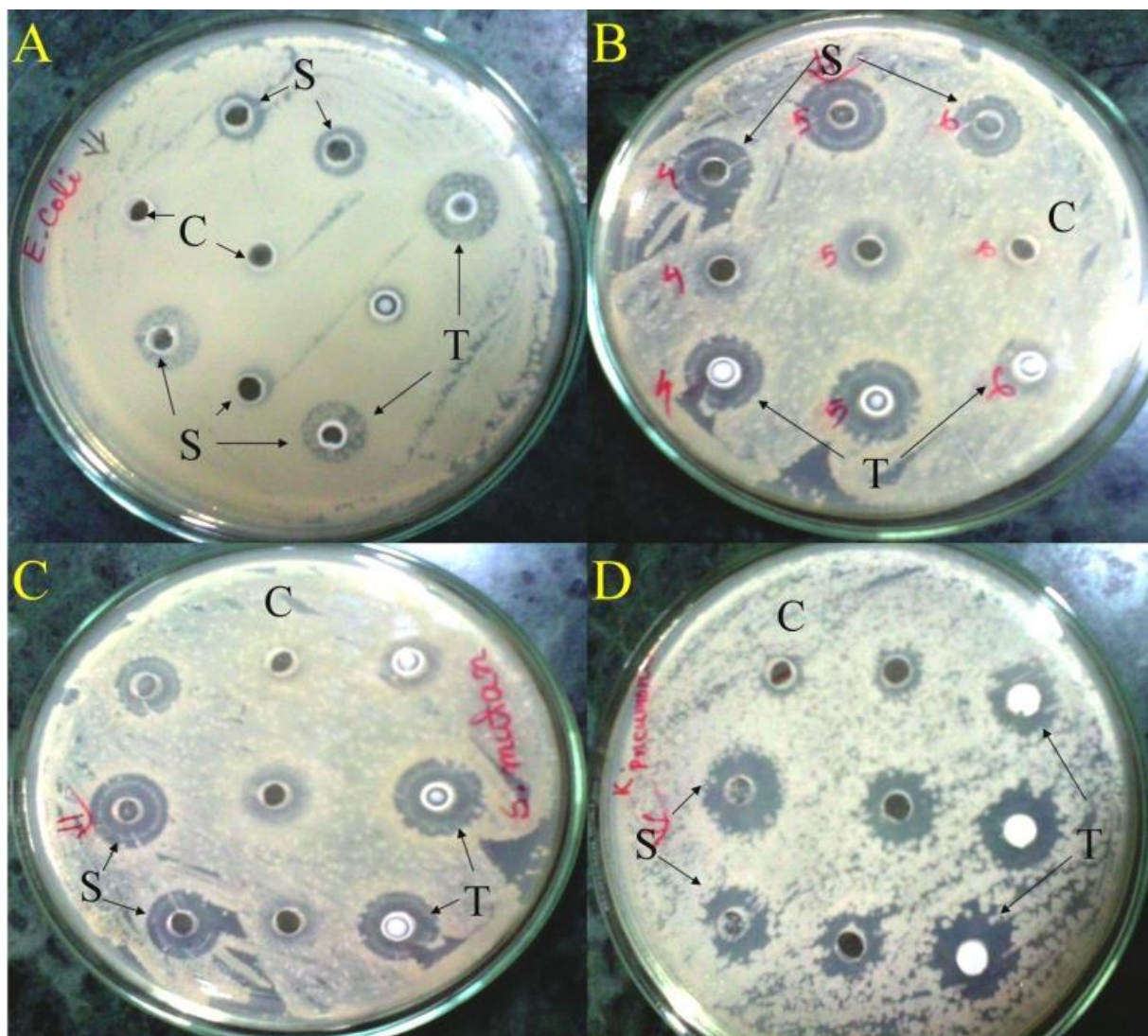


Fig. 5. Antagonistic activity against four Enteropathogenic bacterial Strains – (A) *E. coli*, (B) *B. subtilis*, (C) *K. pneumoniae* (D) *S. mutan*.
C: Control: T: crude curd: S: Cell free supernatant.

CONCLUSION

Novel insights into the metabolism of LAB propose perspectives for the application of a new generation novel starter cultures. Functional LAB starters may offer several health, marketing, and technological advantages. However, fundamental and applied research is still required to optimally execute functional starter cultures in the existing production technology and to obtain quantitative data. Mathematical analysis of the kinetics of functional starter cultures may give up valuable information about the relationship between the food environment and bacterial

functionality, and may contribute to optimal strain selection and process design. This may result in better process control, enhanced food safety and quality, and reduction of economic losses.

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