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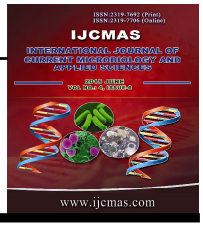

Original Research Article

Identification and Characterization of Folic Acid Producing Potential Starter for Curd Fermentation

Chudar Kodi*, K.M.Gothandam and Geetha Prabakaran

High Throughput Screening Lab, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

Folate producing lactobacillus species Lcf10 isolated from fermented milk products. The strain was evaluated for probiotic properties. It showed 42 % viability at highly acidic condition (pH 3.5), 70% viability in the presence of synthetic gastric juice and 57% survivability at 0.3% of bile concentration for 24 hours. It was susceptible to some antibiotics which reduce the prospect to offer resistance determinant to other organisms if administrated in the form of probiotic preparation. It showed antimicrobial property activity again enteric pathogens like *E.coli, Staphylococcus aureus, Proteus mirabilis, Bacillus cereus*. Probiotic microorganism has been increasing included in various types of food products, especially in fermented milk. Using Lcf10 in curd fermentation proved better survivability of the culture and folic acid production when compared with commercial curd. Incorporation of Lcf10 in curd production will help in producing cheaper and healthier functional food which holds promise to reach mass.

Introduction

Folic acid [also known as vitamin B_9 (Ural, Serdar H. (2008-11) vitamin B or folacin] and folate (the naturally occurring form), as well as pteroyl-L-glutamic acid, pteroyl-L-glutamate, and pteroylmonoglutamic acid are forms of the water soluble vitamin B9. Folic acid is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver (Bailey and Ayling, 2009).

Vitamin B_9 (folic acid and folate inclusive) is essential to numerous body function. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in biological reactions involving folate (Weinstein *et al.* (2003). It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy, as well as in feeding some cancer. While a normal diet also high in natural folates may decrease the risk of cancer, there is diverse evidence that high folate intake from supplementation may actually promote some cancers as well as

precancerous tumors and lesions. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia.

This deficiency can result in many health problems, the most notable one being neural tube defects in developing embryos. Common symptoms of folate deficiency include diarrhea, macrocytic anemia with weakness or shortness of breath, nerve damage with weakness and limb numbness (peripheral neuropathy), pregnancy complications, mental confusion, forgetfulness or other cognitive declines, mental depression, sore or swollen tongue, peptic or mouth ulcers, headaches, heart palpitations, irritability, and behavioral disorders.

Low levels of folate can also lead to homocysteine accumulation (Weinstein *et al.,* 2003) DNA synthesis and repair is impaired and this could lead to cancer development (Weinstein *et al.,* 2003). Supplementation in patients with ischaemic heart disease may also lead to increased rates of cancer.

Probiotics

The term 'probiotic' is derived from Greek word 'pro' means 'life' and has had several meaning over the years.

Lilly and Stillwell introduced the term probiotic describe growth promoting factors produced by the microorganisms. According to Parker (1974), probiotics are organism's substances, which contribute to intestinal microbial balance.

In 1989 was first systematically define them as "a live microbial feed supplement, which beneficially affects the host for improving its intestinal balance". Many microbes as probiotic property, it mainly consist of lactic acid bacteria such as *Lactobacilli, Streptococci, Enterococci, Lactococcus, Bifidobacteria* and also some *Bacillus* sp. as well as some yeast like *Saccharomyces* spp and mold like *Aspergillus* spp.

Probiotic are generally taken either in a form of food or a non-food format. Probiotic are potentially boost the immune system and help in treating conditions like lactose intolerance, diarrhea, colitis, hypertension, cancer, constipation, food allergies, inflammatory bowel diseases etc.

Basic mechanism of probiotics

The key mechanisms by which probiotics may protect the host against intestinal disease include Production of inhibitory substances, Blocking of adhesion sites, Degradation of toxic receptor. Competition for nutrients with pathogenic bacteria, Immune modulation of nonspecific host resistance, Effects on stimulation of systemic immunity.

Materials and Methods

Collection of sample

Curd samples (3 numbers) were collected in sterile vials from different region in vellore. Commercial products like milk and curd were also collected and screened for folic acid production.

Isolation of bacterial strain

Bacterial strain was isolated from the fermented milk and its products by standard microbiological techniques. The samples were collected and serially diluted using 0.85% saline. A 100 µl of the respective dilution was spread on the MRS agar plates and incubated at 37º C for 24–48 hours. Fourteen Colonies with different morphologies were selected at random and further purified by subsequent streaking on MRS agar medium.

Biochemical characterization of lactic acid bacteria

All the fourteen strains were selected for biochemical characterization by Ulrich *et al.* (1950) method. All the strains were subjected to gram staining

Gas production from glucose

Gas production from glucose was assayed by inoculation of cultures into 5ml of MRS broth containing inverted Durham's tubes and incubated at 37ºC for 24 hours (Ravindran Girija and Aswathy, 2008).

Homofermentative Lactic acid bacteria

Lactic acid bacteria ferment glucose with lactic acid as the primary by product. Homofermentative LAB includes *Lactococcus* spp, *Streptococcus, Enterococcus, Pediococcus* and *Aerococcus.*

Heterofermentative Lactic acid bacteria

Lactic acid bacteria ferment glucose with lactic acid, ethanol/acetic acid and carbon dioxide as by product. Heterofermentative LAB includes *Leuconostoc* spp (Gram positive cocci) and Gram positive rods such as *Lactobacillus brevis, Lactobacillus fermentum* and *Latobacillus reuteri, Lactobacillus plantarum, Lactobacillus casei and Lactobacillus curvatus.*

Growth at different temperature

The culture was inoculated into MRS broth and incubated at different temperature like 37ºC and 45ºC (Pedersen *et al.,* 2003).

Growth at different % Nacl

The MRS broth was supplemented with different % Nacl like 2 gram, 4 gram and 6.5 gram of Nacl. The cultures were inoculated into MRS broth which is supplemented with Nacl and incubated at 37ºC for 24-48 hours (Pedersen *et al.,* 2003).

Catalase test

The isolates were tested for the presence of catalase enzyme. The cultures were grown in MRS broth for 24 hours and 100µl of the culture was transferred to the glass slide contains 3% of hydrogen peroxide.

Due to the addition of hydrogen peroxide, catalase positive organisms show air bubbles whereas catalase negative do not show air bubbles (Ravindran Girija and Aswathy, 2008).

Studies on enzyme activity

Starch hydrolysis

All the isolates were screened for their ability to hydrolysis starch. Organisms were inoculated in the starch agar and incubated at 37ºC for 1 week. Amylase production was checked by pouring iodine on the plates and observed for clear zone (Taiwo, 2010).

Protein hydrolysis

All the isolates were screened for their ability to hydrolysis protein.

The cultures were inoculated into MRS agar which is supplemented with 10% skim milk agar and the inoculated cultures were incubated at 37ºC for 24-72 hours. Colonies were observed for clear zone (Ravindran girija and Aswathy, 2008).

Screening for lipase enzyme

All the isolates were screened for their ability to produce lipase enzyme. The

cultures were inoculated into MRS agar which is supplemented with 1% 0f tributyrin glycerol and the inoculated cultures were incubated at 37ºC for 2 days. Colonies were observed for opaque zone (Mohd Yusof *et al.,* 1988).

Screening for folate production

The folate producing ability of the isolates was determined by inoculating into folic acid casei medium. Folic acid casei medium is an assay medium contains all the ingredients and essential nutrient for the growth of the test organism except the material under study.

All the isolates were inoculated into folic acid casei medium. Aerobic growth conditions were obtained by incubating the inoculated broth under continuous shaking of 210 rpm at 30ºC for 18–24 hours. The 18 h old culture was subjected further for HPLC analysis.

Folate analysis by high pressure liquid chromatography (HPLC)

Preparation of standard

Stock solution (1mg/ml) of folic acid was prepared by dissolving 1mg of folic acid (Himedia) in 1ml of milliQ water. Working solution was prepared by dissolving 10 microlitre of the stock solution into 90 microlitre of milliQ water.

Sample preparation for HPLC analysis

Folate estimation was done using HPLC. 1ml of the culture was taken and sonicated at 50,000 Hz for 2 minutes with a pulse of 2 seconds. The sonicated sample was subjected to heat treatment at 100º C for 5 minutes to release any folate bound to folate binding proteins. To prepare cell free extract, the sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant was taken for folate analysis.

Chromatographic condition

The chromatographic analysis was performed using shimadzu which consisted of LC 20-AT pump and SPD-20A detector. The column used was a Luna 5µ C18 100A with a dimension of 250×4.60 mm and the packing size of 5 micron.

Freshly prepared mobile phase consisted of a mixture of 75 ml of milliQ water and 0.5ml of glacial acetic acid and pure HPLC grade methanol in the ratio of 70:30. The flow rate was maintained at 1 ml/min, the column temperature was maintained at 40º C and the sample was detected at 282 nm.

Evaluation of probiotic properties
Acid tolerance

Acid tolerant ability of the isolates was confirmed by viable count method (Gowri Sukumar and Asit Rajan Ghosh, 2010). One ml of the isolates grown in the MRS broth and the optical density were adjusted to of 0.280 at 600 nm. one ml of the culture were inoculated into MRS broth whose pH was adjusted to 3.5 with 1N HCL and the cultures were serially diluted using 0.85% saline and a 100µl of respective dilution was spread on the MRS medium and incubated at 37ºC for 24–48 hours as 0^{th} hour inoculation.

All the culture tubes were incubated at 37º C for 4 hours. The viable bacteria were enumerated by plating 100µl of culture on MRS medium and incubated at 37º C for 24 hours as 4^{th} hour inoculation. The growth of the bacterium was expressed in colony forming units per milliliter (log CFU 4^{th} hour/ log CFU 0^{th}) ×100 and the percent survival of strain was then calculated.

Bile salt tolerance

Bile tolerant ability of the isolates was determined by inoculating 1ml of the cultures in 9ml of MRS broth supplemented with 0.3% bile salt and the cultures were serially diluted and 100µl of the culture was spread on MRS medium and incubated at 37º C for 24–48 hours as 0^{th} hour inoculation. All the culture tubes were incubated at 37º C for 24 hours.

The viable bacteria were enumerated by plating 100µl of culture on MRS medium and incubated at 37 ºc for 24 h.

Growth of the bacteria was expressed in colony forming units per milliliter (log CFU of 24^{th} hour/log CFU of 0^{th} hour)×100 and the percentage of survivability of the strains was then calculated (Gowri sukumar and Asit Rajan Ghosh, 2010).

Gastric juice tolerance

Survival in synthetic stomach juice is the most important probiotic property. The synthetic juice was prepared using 8.3g of proteose peptone, 3.5g of glucose,2.05g of NaCl,0.6g of KH_2PO_4,0.4g of $CaCl_2$, 0.37g of KCl, 0.05g of bile, 0.1g of lysozyme and 13.3mg of pepsin dissolved in 1 litre of milliQ water and the pH was adjusted to 2.5 with 1M HCL.

The gastric juice was heated at 37º C for 30 minutes and it was filtered using a syringe filter in a sterile manner before use. To the test tubes containing 10 ml of juice, 100µl of culture was added (Pedersen *et al.,* 2003).

Samples were taken at 0,30 and 180 minutes and the survival rate was measured by spreading 100µl of different dilution on MRS agar plates and incubated anaerobically at 37 ºC for 48 hours.

Quantitative estimation of organic acid

The 24 hours lactic acid bacteria cultures were centrifuged at 10,000 rpm for 10 min at 4º C and the supernatant were used for quantitative estimation of the organic acid. Few drops of phenolphthalein were added as an indicator to 1ml of the supernatant and it was titrated against 0.1N NaOH. It has been standardized that 1ml of 0.1N NaOH is equivalent to 90.08 mg of lactic acid (A.O.A.C). Amount of lactic acid produced was calculated from the standard (Gowri sukumar and Asit Rajan Ghosh, 2010).

Screening for the bacteriocin production of the isolates

Antibacterial activity was determined by Agar well diffusion method. This involved seeding the petridishes containing Muller Hinton agar with the reference strain in the log phase. The specific LAB strain was inoculated aseptically in sterile MRS broth medium and incubated at 37◦C for 18 hours. The 18 hours culture was centrifuged at 10,000 rpm for 5 minutes. 60 µl of the supernatant was aseptically transferred to the well and the plates were incubated at 37º C for 24 h and growth inhibitions of the pathogens were observed (Gowri sukumar and Asit Rajan Ghosh, 2010).

Antibiotic susceptibility test

Antibiotic drug susceptibility was determined by swabbing the test organism on the Mueller Hinton agar and the standard antibiotic discs were placed on the surface of the agar medium. Plates were observed for the zone of inhibition after 24 h of incubation at 37 ºC (Gowri sukumar and Asit Rajan Ghosh, 2010).

Potential effects of using folate producing probiotic lactobacilli as starter for curdling process

Probiotics are live microbial feed or food supplements, which beneficially affect the host by improving its intestinal microbial balance. The cells were routinely propagated on 2 successive days, by 1% inoculums in MRS broth and incubated at 37 ºC for 24 h. The optical density and the CFU of the starter organisms were measured. The cell mass was harvested by centrifugation at 10,000 rpm for 10 minutes. After centrifugation, the cell pellet were collected and inoculated in milk at different percentage to see vigorous propagation of the starter organism. The inoculated milk was incubated at room temperature until it gets ferment (Frederic Leroy and Lucde Vyst, 2000).

Folate analysis in curd by HPLC

Preparation of curd sample for HPLC analysis

Curd sample were analyzed for folate by HPLC method. To prepare cell free extract, Curd sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected and mixed with the mobile phase for HPLC analysis.

Results and Discussion

Lactobacillus creates a healthy and beneficial environment and protect from potentially harmful microorganism in the gut ecosystem when they are present in sufficient numbers.

The fermented milk is a good source for the growth of lactic acid bacteria. Fourteen different colonies were selected lcf1, lcf2, lcf3, lcf4, lcf5, lcf6, lcf7, lcf8, lcf9, lcf10, lma1, lma2, lma3 and lca1 based on different morphology and named based on the source. Biochemical characterization of the isolates is given in Table 1 & 2.

Studies on enzyme activity

Starch hydrolysis

Starch hydrolysis test proved that none of the isolates are amylolytic. There are reports of amylase production by LAB group. Since curd contains disaccharides, amylase production ability was not found in the isolates.

Protein hydrolysis

All the isolates were screened for their ability to produce an enzyme called protease. Among 14 isolates, lcf2, lcf3, lcf4, lcf5, lcf6, lcf8, lcf9, lcf10 and lma1 isolates had the ability to produce protease which is confirmed by the clear zone formation.

The isolation source may be the reason for protease production of the isolates. However further test is required for confirmation

Screening for lipase enzyme

All the 14 isolates gave negative result in lipase production as expected.

Folate producing ability of the isolates

Fourteen strains were subjected to screen for their ability to produce folic acid by growing them in the chemically defined medium short of folic acid in the folic acid casei medium incubated at 30º C under continuous shaking (210rpm).

Five strains (lcf7, lca1, lma1, lma2 and lma3) were not able to survive in the chemically defined medium short of folic acid exhibiting their inability to synthesize the vitamin. Folic acid production in the strains was analyzed by hplc.

HPLC analysis of the isolates for folate production

The strains lcf7, lma1, lma2, lma3 and lca1 didn't produced folic acid which has been proved by HPLC result on comparison with the retention time of standard folic acid. lcf1, lcf2, lcf3, lcf4, lcf5, lcf6, lcf8, lcf9 and lcf10 are the strains have the capability to produce folic acid. Concentration of folic acid produced was calculated by comparing with the area obtained with standard folic acid retention time and area. Based on the retention time, and area obtained strain lcf10 produced more folic acid compared to rest of the strains and it produce approximately 0.0104 mg/ml.

Studies on probiotic properties

Acid tolerance

The ability of the strains to survive in acidic condition is a very important probiotic property. All the isolates showed viability in highly acidic condition i.e. pH 3.5 except three strains lma1, lma2 and lcf7. The strains lcf6 (90%), lcf1 (80%), lcf3 (70.8%) and lcf10 (42%) showed maximum survivability rate in highly acidic condition. The strains lcf7, lma1 and lma2 were not able to survive in acidic pH. Hence, it indicates these strains were not able to tolerate the acidic pH. Therefore, it indicates these strains lcf7, lma1 and lma2 were not having the probiotic properties.

Gastrointestinal tract has acidic pH; hence tolerance to low pH of these isolates has exhibited good probiotic property.

Bile tolerance

The ability of the strains to survive in bile salt is a very important probiotic property. The strains lcf3, lcf8 and lma2 failed to survive in MRS broth supplemented with 0.3% bile salt. The strains lcf7 (75%), lcf4 (66%) lma1 (66%) and lcf10 (57%) showed maximum survivability in the bile salt which again proved their probiotic ability.

Gastric juice tolerance

Maximum number of the isolates has the ability to survive in the synthetic gastric juice. lcf2, lcf3, lcf7, lcf10, lma1 and lma2 are the strains failed to survive in the synthetic gastric juice with pH 2.5. Hence it indicates these strains could not survive in gastrointestinal tract.

Probiotic potential of isolates

Determination of tolerance of the isolates for intestinal conditions is given in tables 3–5.

Quantitative estimation of organic acid

All the isolates have the capability to produce organic acid such as lactic acid of varying concentration after 24 hours of incubation at 37ºC. The lactic acids produced by the organisms were estimated by titrating against 0.1N NaOH. The isolates lma1, lma2 and lcf9 produced more organic acids. Isolate lma2 produced the maximum lactic acid approximately 158.55 mg/ml, lcf10 produced 97.84 mg/ml and was indeed the most tolerant to acidic pH. Isolate lcf7 produced least amount of lactic acid approximately 63.42 mg/ml compared to the other strains

Screening for the bacteriocin production of the isolates

Survivability of the strains in the intestinal tract needs the probiotic organism to be competitive. Hence antimicrobial of the isolates against common intestinal pathogens was attempted. All the strains acted against enteric pathogens which imply bacteriocin production of the strains. Most

of strains showed zone of inhibition against *E. coli, proteus mirabilis, Bacillus cereus.*

Antibiotic susceptibility testing

Antibiotic resistant is an additional property of probiotic. Organisms resistant to antibiotic will not be washed away if antibiotic is taken. Most of the isolate were sensitive to chloramphenicol, tetracycline, bacitracin, gentamycin, methicillin, erythromycin, kanamycin, rifampicin and torbramycin. Most of the strains were resistant to antibiotics like penicillin, amphicillin and vancomycin.

Folate producing potential starter for curd preparation

The best folate producing probiotic strains such as lcf1 and lcf10 were used as starter organisms for curdling process. The CFU of the starter organism was measured as 2.7 for lcf10 and the CFU for lcf1 was measured as 2.2. The culture samples were centrifuged at 10,000 rpm for 10 min at 4ºC and the media components free pellet were inoculated in to milk. The different percentage of inoculums such as 10%, 20%, 30% was added to the milk to measure the vigorous propagation of the starter organisms and to optimize the inoculum size of the starter organisms. The fermentation period for curdling process was measured.

Table.1 Gram reaction, colony morphology and gas production

strains	Gram reaction	Colony morphology	Gas production	
			homo	Hetero
Lcf1	Gram+ short rods	Creamy white, convex colonies.	_	+
Lcf2	Gram+ rods	Creamy white, mucoid colonies.	_	+
Lcf3	Gram+ short rods	White colour, entire margin.	_	+
Lcf4	Gram+ rods	White colour, entire margin,mucoid colonies.	_	+
Lcf5	Gram+ rods	Cream colour colonies with entire margin.	_	+
Lcf6	Gram+ short rods	Cream colour colonies with entire margin.	_	+
Lcf7	Gram+ lengthy rods	White colour rough colonied.	+	_
Lcf8	Gram+ short rods	Cream colour mucoid colonies.	_	+
Lcf9	Gram+ rods	Creamy white mucoid colonies with entire margin.	_	+
Lcf10	Gram+ rods	Creamy white,entire margin,convex colonies.	_	+
Lma1	Gram+ coccobacilli in pairs	White colour pin point colonies.	+	_
Lma2	Gram+ coccobacilli in pairs	White colour pin point colonies.	+	_
Lma3	Gram+ cocci in pairs	White colour pin point colonies.	+	_
Lca1	Gram+ lengthy rods in chains	White colour pin point colonies.	_	+

Table.2 Growth at different temparature and different % NaCl

strains	Growth at different temp		Growth at different % Nacl			catalase
	37°	45°	2%	4%	6.5%	
Lcf1	+	+	+	+	+	_
Lcf2	+	+	+	+	+	_
Lcf3	+	+	+	+	+	_
Lcf4	+	+	+	+	+	_
Lcf5	+	+	+	+	+	_
Lcf6	+	+	+	+	+	_
Lcf7	+	+	+	+	+	_
Lcf8	+	+	+	+	+	_
Lcf9	+	+	+	+	+	_
Lcf10	+	+	+	+	+	_
Lma1	+	+	+	+	+	_
Lma2	+	+	+	+	+	_
Lma3	+	+	+	+	+	_
Lca1	+	+	+	+	+	_

Table.3 Acid tolerance of the isolates

Strains	Acid tolerance		
	0^{th} hour	4^{th} hour	Percentage of survivability
Lcf1	326	124	80%
Lcf2	750	22	46%
Lcf3	274	62	70.8%
Lcf4	436	19	46%
Lcf5	546	27	51%
Lcf6	113	87	95%
Lcf7	0	0	0
Lcf8	346	15	44%
Lcf9	530	12	37%
Lcf10	436	55	42%
Lma1	0	0	0
Lma2	0	0	0

Table.4 Bile tolerance of the isolates

Strains	Bile tolerance		
	0^{th} hour	24^{th} hour	Percentage of survivability
Lcf1	4	2	50%
Lcf2	36	4	40%
Lcf3	11	0	0
Lcf4	9	4	66.6%
Lcf5	7	2	37%
Lcf6	14	2	27%
Lcf7	7	4	75%
Lcf8	1	0	0
Lcf9	20	2	23%
Lcf10	6	3	57%
Lma1	5	3	66%
Lma2	2	1	0

Table.5 Gastric juice tolerance of the isolate

Strains	Synthetic gastric juice tolerance		
	0^{th} hour	30 minutes	180 minutes
Lcf1	Tntc	Tntc	531
Lcf2	10	0	0
Lcf3	23	0	0
Lcf4	91	2	1
Lcf5	510	221	1
Lcf6	360	30	29
Lcf7	1	0	0
Lcf8	520	210	2
Lcf9	7	1	0
Lcf10	527	288	55
Lma1	0	0	0
Lma2	0	0	0

Table.6 Antibacterial activity of the isolates

Strains	*E.coli*	*Staph.aureus*	*B.cereus*	*Proteus.mirabilis*
Lcf1	16mm	18mm	11mm	11mm
Lcf2	14mm	15mm	12mm	14mm
Lcf3	15mm	15mm	No zone	14mm
Lcf4	13mm	No zone	No zone	16mm

Lcf5	15mm	No zone	11mm	15mm
Lcf6	16mm	No zone	No zone	14mm
Lcf7	12mm	21mm	10mm	13mm
Lcf8	12mm	No zone	12mm	13mm
Lcf9	13mm	No zone	12mm	13mm
Lcf10	16mm	13mm	No zone	12mm
Lca1	No zone	No zone	No zone	No zone
Lma1	15mm	11mm	11mm	12mm
Lma2	13mm	10mm	10mm	12mm
Lma3	No zone	No zone	No zone	No zone

Table.7 colony forming units of the starter culture

strains	**Log CFU**	**OD at 600nm**
Lcf1	2.2	2.1
Lcf10	2.7	>2.5

Table.8 Starter culture-Comparison of microbial population in commercial curd and starter curd

Curd sample	**Total bactetria Log CFU**	**Lactic acid bacteria Log CFU**	**Yeast Log CFU**
Control curd	2.65	2.12	1.9
Commercial curd	2.62	2.77	2.5
Lcf1 10%	2.37	1.46	Nil
Lcf1 20%	2.6	1.65	Nil
Lcf1 30%	2.6	1.73	Nil
Lcf10 10%	2.13	1.36	Nil
Lcf10 20%	2.59	1.8	Nil
Lcf10 30%	2.6	1.8	Nil

Table.9 Analysis of fermentation changes effected by different inoculum size of starter

Curd sample	**Fermentation time**	**flavor**	**Organic acid(mg/ml)**	**pH**
Control	-	Bad	72.48	3.92
Commercial curd	-	Good	108.72	4.58
Lcf1 10%	12 hours	Good	45.3	4.49
Lcf1 20%	10 hours	Good	63.42	4.19

Lcf1 30%	8 hours	Good	81.54	4.07
Lcf10 10%	10 hours	Good	54.36	4.36
Lcf10 20%	8 hours	Good	81.54	4.23
Lcf10 30%	7 hours	Good	108.72	4.06

Figure.1 HPLC analysis of folic acid produced by strain lcf10

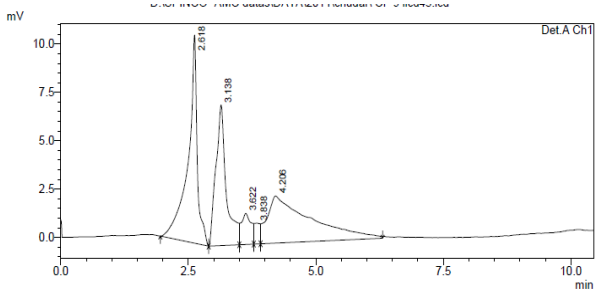
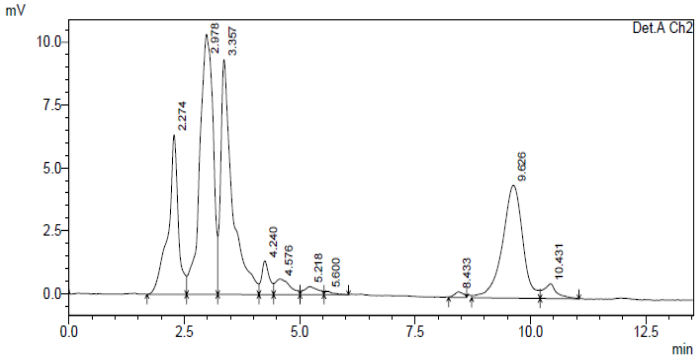


Figure.2 HPLC analysis of folic acid in curd produced by 30% innoculam of lcf10



The viable count of the starter organisms in the curd sample was calculated using microbiological techniques such as serial dilution. The 30% inoculum size of Lcf10 fermented the milk within 7 hours compared to other isolates and it produced more folic acid in the curd. The strain Lcf10 provided good flavor and consistency in curd. It produced more organic acid and showed a pH of 4.06.

Folate analysis in curd using HPLC

The amount of folate present in the test curd and the control sample were analyzed using High Performance Liquid Chromatography. The strain lcf10 produced more folic acid compared to lcf1.The strain lcf10 at 30% of inoculum size produced more folic acid approximately 0.02mg/ml compared to the strain lcf1 which produced 0.015 mg/ml and to that of commercial curd sample which contains 0.01 mg/ml. Hence curd produced using starter culture LCF10 had more folic acid content than commercial curd

Summary and conclusion

Curd sample was collected from different locations of vellore and processed for microbiological analysis. 14 Lactic acid bacteria isolates were selected depending on colony morphology and tested for folic acid producing ability. In addition all the isolates

were analyzed for additional beneficial properties and enzyme activities. LCF10 and LCF1 were selected as best folate producer and exhibited best probiotic activity. Hence they were used as starter culture in curd development. Fermentation conditions and inoculum size was optimized for folic acid production and curdling process. LCF10 inoculated proved to be the best starter at 30 % inoculum concentration having best flavor, reduced fermentation time and highest folic acid production. LCF 10 inoculated curd found to be superior when compared with commercial curd.

Hence we conclude that incorporation of Lcf10 in commercial curd production will help in producing cheaper and healthier functional foods which holds promise to reach masses.

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