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Formulation of Culture Medium for the Enumeration of Lactic Streptococci and Lactobacilli from Fermented Milk Products

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Authors' contributions

This work was carried out in collaboration among all authors. Author GS conceived, designed the analysis, collected the samples, performed the microbiological analysis of the study and wrote the first draft of the manuscript, Authors SCS, YS assisted in literature search and statistical analysis while Author PR supervised all the study protocols and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study is focused to formulate a single selective differential medium for the enumeration of mixed lactic flora such as lactic streptococci and lactobacilli in domestic and market fermented milk products.

Study Design: In order to enumerate lactic streptococci and lactobacilli in a mixed flora of fermented milk, formulation of single selective medium was optimized using inhibitors and indicator instead of separate selective medium for lactic streptococci and lactobacilli.

Place and Duration of Study: The present study was conducted in the Department of dairy Microbiology, Dairy Science College, Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Hebbal, Bengaluru-24, Karnataka from February 2020 to February 2021.

Methodology: Fermented milk samples like curd, yoghurt were collected, serially diluted and pour plated using Readymade M17 and MRS agar media and formulated selective yeast glucose agar medium. Lactic colonies obtained were enumerated, expressed as $\log_{10}$cfu/g and critical difference was calculated to declare significance and non-significance among the microbial parameters.

Results: Readymade M17 and MRS agar media gave better recovery of viable cells of lactic streptococci and lactobacilli from market samples of curd, yoghurt and domestic curd samples. The study helped to convert a general purpose medium yeast glucose agar as selective differential medium with addition of calcium propionate of 0.8 per cent to avoid *Bacillus* spore formers and sodium benzoate of 1.2 per cent to inhibit yeast, the common contaminants in fermented milk with 0.05 per cent BCP that led to formation of purple colonies of lactic streptococci and yellow colonies of lactobacilli.

Conclusion: The selective yeast glucose agar with 0.8 per cent and sodium benzoate of 1.2 per cent with 0.05 per cent BCP with purple colonies of lactic streptococci and yellow colonies of lactobacilli can be successfully used. This study verified the enumeration of mixed lactic flora in fermented milk products instead of M17 medium for lactic streptococci and MRS for lactobacilli, separately

Keywords: Bacillus spore formers; bromocresol purple; lactic streptococci; yeast; yogurt.

1. INTRODUCTION

"Lactic acid bacteria (LAB) are among the most important groups of microorganisms used in food fermentation. LAB are a group of Gram positive, non-spore forming cocci or rods, catalase negative and fastidious organisms with high tolerance to pH" [1]. However, some species of the Lactobacillus genus can grow in pH varying between 4.5 and 6.5, whereas, some strains can grow in more lower pH values.

"Fermentation of milk, preserves its nutrients for long time and fermentation is made possible by inoculating the milk with lactic acid bacteria. Fermented milk products extend potential therapeutic benefits to the consumers. People have the concept of using fermented milk products as they are safe for consumption due to end products of fermentation by LAB due to their well-known status as GRAS (Generally Regarded As Safe). LAB include a variety of industrially important genera such as *Lactococcus, Streptococcus, Leuconostoc, Pediococcus* and Lactobacillus. LAB play an important role as starter cultures for fermentation in dairy and food industries. Starters are selected strains of microorganisms (LAB) deliberately added to milk during conversion into dahi, yoghurt, cheese and other fermented dairy products. The LAB could be mainly divided into two groups based on the end-products formed during the fermentation of glucose. Homofermentative lactic acid bacteria such as *Lactococcus* spp., *Streptococcus thermophilus*, and some of the lactobacilli like *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lb. acidophilus* produce lactic acid as the major or sole end product of glucose fermentation. Where as the heterofermentative group produce lactic acid, other acids and even gases and include *Leuconostoc* spp.*, Lb. brevis, Lb. fermentum"* [2].

"Enumeration of mixed lactic flora like lactic streptococci and lactobacilli may be difficult using a single culture medium though selective M17 and MRS agar or general-purpose medium like yeast glucose agar or Elliker agar media present. Eight agar media and one control medium were analyzed to compare their proficiency in evaluating the *Lactococcus* population in raw milk: M17 Nal, Elliker, modified Elliker, PCA (plate count agar) + milk, turner agar modified KCA, modified Chalmers, Turner, FSDA. (fast

slow differential agar). The M17 medium was used as reference. The KCA medium proved to be the most selective towards lactococci and it was not possible to separate the *Streptococcus* from the *Lactococcus* colonies on KCA. The "*Lactococcus*-like" population including these two genera was estimated at a mean level of 3.18 log(cfu)/mL and 4.14 log(cfu)/mL in cow and goat raw milk, respectively" [3]. "A load of *Lactococcus* spp. was counted as 1.12×10^7, $8.01\times10^7$and 2.75×10^9 CFU/ml from raw cow's milk, cheese and yogurt, respectively, on M17 agar" [4].

BRIGGS [5] "a medium for streptococci and lactobacilli, LAE (Lactic-Agar-Elliker), were developed" [6]. "A number of lactobacilli strains did not grow well in any of these media, so a nonselective medium known as lactobacilli MRS that was able to support the growth of lactobacilli was developed" [7]. "Briggs agar was a medium frequently used for the cultivation of lactobacilli from milk and dairy products. Early observations suggested that typical flat greyish-brown and rough colonies ("B-colonies") appearing on Briggs or BL agar plates may be *L. acidophil*us. MRS medium had a similar composition to APT (All-purpose Tween 80 agar) and was developed primarily with the intention of substituting tomato juice by defined growth factors such as Mg^{2+} and Mn^{2+}. De Man Rogosa Sharpe containing bile (MRSB), Man-Rogosa Sharpe agar (MRS) containing nalidixic acid, paromomycin, neomycin sulphate and lithium chloride (MRS-NPNL), M17 and *L. casei* (LC) agar failed to select *Lactobacillus acidophilus, Bifidobacterium,* starter LAB and *L. casei* strains respectively. However, LC agar appears appropriate for *L. paracasei* and MRSB for yoghurt starter bacteria in the absence of *L. reuteri* and *L. rhamnosus"* [8]. "The selective potential of culture media largely depended on target species" [9]. "MRS agar added with soya peptone of 0.02, glucose of 0.12, yeast extract of 0.2 and magnesium sulphate of 0.08 per cents to normal composition and they found good growth with counts of 9 $\log_{10}$cfu/ml of lactobacilli. Both MRS and Acetate agar statistically showed significant difference in counts of lactobacilli from yogurt sample by serial dilution using PBS (phosphate buffered saline) as diluent with log counts of 7.41 and 4.59 cfu/g respectively when incubated the plates at 37°C in candle jar" [10]. "During the cultivation of milk samples during the first, third- and seventh-days, lowest number of lactobacilli colonies per day was recorded during the seventh day, at 3.7 ± 0.1 log cfu/mL in cow milk at 37 °C for 24–72 h using MRS medium" [11].

"It was found that MRS agar medium was not efficient in distinguishing between *Lactobacillus rhamnosus* LV108 and *Streptococcus thermophilus* grx02. In co-cultured milk (*Lb. rhamnosus* LV108+ *Str. thermophilus* grx02), the viable count of *Str. thermophilus* grx02 could be enumerated by controlling the culture time on LM17(Lactobacillus selective agar) up to 10^8 cfu/ml. By restricting the carbon source and culture time, mixtures of *Lb. rhamnosus* LV108 and *Str. thermophilus* GRX02 could be enumerated rapidly and easily in 48 h (minimum 36 h), which could benefit the development, production and storage of probiotic dairy products containing these two species" [12].

"If only one lactic culture was used in fermented milks, the problem to enumerate would be less. But the problem encountered to enumerate in a mixture of lactic cultures used in the preparation was found to be difficult. The viability of each group of lactic culture might be a problem Tomato juice found to stimulate the growth of many LAB and it was included in Briggs agar" [5]. "The yogurt starters and commercial samples that grew on Elliker's lactic agar supplemented with 1% Tween 80 and 50 mg/ml of 2,3,5-triphenyltetrazolium chloride produced small, red *Streptococcus thermophilus* colonies and larger, white *Lactobacillus bulgaricus* colonies. The distinction was somewhat strain dependent but was satisfactory in most cases. Addition of 7 % skim milk (11% solids) to lactic agar in place of 2,3,5- triphenyltetrazolium chloride allowed good rod-coccus differentiation, regard- less of strain or yogurt brand [13]. A total of 10 sucuk (Turkish-type fermented sausage) samples, obtained from Denizli, had on an average of 8.34 log CFU/g TAMB, 8.91 log CFU/g LAB (at the MRS agar) and average 8.25 log CFU/g LAB (at the Elliker's lactic agar) both lactobacilli and lactic acid producing cocci" [14]. "The plate count agar with bromocresol purple was recommended for the enumeration of lactic acid bacteria (LAB) in foods was good for enumeration but not for differentiation of each LAB in a mixed culture. Compared with PCABCP, mMRS produced larger colonies of various sizes in 2 days, making enumeration easier. However, various LAB produced colonies with the same white colour, made it difficult to differentiate each species. Compared with PCA-BCP and mMRS, mMRS-BPB could differentiate each species based on its characteristic pH change during growth.

Because BPB changed colour within a range from pH 3 to 5, it was useful for detection of the pH change produced during fermentation of LAB" [15]. "The Fast-Slow Differential Agar (FSDA) medium was developed in 1984 and still remains the standard to rapidly differentiate fast and slow milk-coagulating lactic streptococci but unable to selectively isolate fast acid-producing strains due to the presence of a diverse microbiome including Non-Starter LAB and spoilage Gram-negative microbiota modified FSDA (mFSDA) with increased selectivity of nalidix acid (inhibit Gram negative bacteria), ascorbic acid and yeast extract stimulate the growth of lactic streptococci and the pH indicator bromocresol purple enabled the chromogenic discrimination between LAB with different acid production capability" [16,17].

"Sodium azide was added to inhibit the contamination flora, including Gram negative flora and purple bromocresol allowed a direct selection of the Gram + and lactose + flora. For all samples medium M3 appeared to be the most appropriate for growth of Gram + and catalase – bacilli, owing to the high growth rates and the colony diameters ranging from 1-2 mm" [18]. "LC (*Lactobacillus casei*) agar appeared appropriate for *Lactobacillus paracasei* and MRSB (MRS with bile) for yoghurt starter bacteria *Latobacillus delbrueckii* ssp. *bulgaricus* or *S. thermophilus* and LC agar could be used to target *Lb. paracasei*. Selective recovery of *Lb. delbrueckii* ssp*. bulgaricus* could be achieved using MRS 5.2 incubated at 45ºC anaerobic for 72 h. In general, MRS 5.2 at 45 °C for 72 h supported the growth of almost all the *Lactobacillus* spp with viable count of more than 9 log" [8]. "Yoghurt samples showed higher counts of LAB that ranged from 1.0×10^6 to 5.6×10^7 cfu/mL and 2.2×10^7 to 5.4×10^8 cfu/ both at aerobic and anaerobic condition using MRS agar (pH 6.2-6.6) and Rogosa agar (pH 5.2- 5.6), respectively for total LAB enumeration as well as lactobacilli isolation.YGLA (Yeast Glucose Lactic agar pH 7.0**)** was used for the isolation of streptococci incubated at 37 °C for 48-72 h both at aerobic and anaerobic conditions. In general, anaerobic LAB growth of 6.1×10^5 to 5.4×10^8 cfu/mL counted higher than the aerobic growth of 1.1×10^5 to 5.6×10^7 cfu/mL" [19]. "The mRCM-aniline blue performed better than the conventional medium in culturing, enumerating, and differentiating *L. bulgaricus*. Therefore, mRCM-blue could be used as a selective medium to enhance the growth and differentiation of *L. bulgaricus* in order to meet the increasing demand for this beneficial species of bacteria [20]. Commercial yogurt such as plain, apple, blue berry and yogurt prepared from heat treated raw milk were subjected for the viable count estimation using BCP (Bromocresol Purple) agar containing 0.5 % poly peptone, 0. 5 % yeast extract, 0.1 % glucose, 0.1 % Tween 80, 0.01 % L-cysteine and 0.006 % bromocresol purple with pH 7.0. The lactic counts of the yogurt samples were 3.7×10^8, 2.7×10^8, 6.7×10^8 and 5.8×10^8 cfu/g for plain, apple, blue berry and yogurt prepared from raw milk, respectively" [21]. "The modified Reinforced clostridial medium (RCM) was used to selectively enumerate and isolate *Lactobacillus delbrueckii* ssp. *bulgaricus,* by optimizing the addition of 0.5 % fructose, 0.5 % dextrose, 1 % maltose, and 0.25 % sodium pyruvate while replacing lactose as a carbohydrate source. The cell recovery and bacterial counts of *Lb. delbrueckii* ssp. *bulgaricus* in tested. products (pure *Lb. delbrueckii* ssp. *bulgaricus* strains, starter culture, probiotic supplements and yogurt) using mRCM with sodium pyruvate (mRCM-PYR) were significantly higher than de Man, Rogosa, and Sharpe (MRS) culture medium. The growth of other lactic acid bacteria (*Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus rhamnosus*, and *Lactobacillus reuteri*) and Bifidobacteria were retarded in this modified medium compared with their growth in MRS. The results thus suggested that mRCM-PYR could be recommended as a reliable alternative growth medium for the selective enumeration and isolation of *Lb. delbrueckii* ssp. *bulgaricus* in a mixed culture" [22].

In this study, it was considered as a basis to define a selective medium, by adding a pH indicator and some inhibitory compounds to select the LAB flora from various dairy products like fermented milk products such as curd, yoghurt etc. "Common microbial contaminants like aerobic bacterial spore formers and yeast if present in fermented milk products posed problem during enumeration and predominated over starter or lactic acid bacteria. There might be requirement of certain inhibitors to be incorporated to medium to overcome the contaminants" [23,24]. "Calcium propionate ($C_6H_{10}CaO_4$) which having MW of 186 was water soluble. The compound was effective at pH 5.5. It prevented *Bacillus* spp. from producing the energy as they were protonophores at 0.4-1 per cent in LAB media. Sodium benzoate (C_6H_5COONa) soluble in water with MW of 144 had its optimum activity at pH 5-6. The concentration of 0.1-0.3 per cent inhibited yeast

and molds which the studies presumed due to leakage of PMF, thus, ATP synthesis of fungi was affected" [23,24]. If fermented milk had yeast as contaminant then yeast predominated over LAB. Once yeast sometimes even *Bacillus* spp. made colonies, then isolation of the LAB became difficult. Media might require addition of antimycotic (sodium benzoate) and anti-sporulating (calcium propionate) agents [25]. The focus of the present study was to formulate a single medium for lactic streptococci and lactobacilli in fermented milk products along with curbing the growth of contaminants such as spore formers and fungi

2. MATERIALS AND METHODS

Samples collected and methods used for enumeration of lab are explained in this section.

2.1 Collection of Various Market and Domestic Fermented Milk Product Samples and for the Enumeration of LAB

Market available samples of curd, yoghurt and domestic curd samples were collected aseptically, direct microscopic count was conducted. Based on DMC, serial dilution of samples were carried out for the enumeration of lactococci, *S.thermophilus* and lactobacilli [26].

2.1.1 Direct microscopic count (DMC)

Samples to be analyzed were mixed well and diluted to 1:10 by using PBS (phosphate buffered saline) as diluent. Sample of 0.01 ml was transferred using a Breed's pipette onto a marked slide of 1 cm^2 and smear was prepared by spreading evenly in the marked area. The smear was fixed using ethanol for 2 min, de-fattened using xylene for 2 min and stained using borax methylene blue for 5min and rinsed with tap water. Then observed under the oil immersion objective and organisms was counted in each of the field. The average number of organisms per field was calculated and multiplied by microscopic factor(MF), dilution factor and 100 expressed as log10/g. MF = Area of the smear (100) divided by Area of the microscopic field (πr^2). To determine the radius of microscopic field finding diameter was important and was done using stage micrometer, each division of stage micrometer was 0.01mm. Once the diameter was determined, converted to radius and calculated using formula πr^2.

2.1.2 Serial dilution technique and pour plating of the samples

After knowing the DMC, market samples of curd, yoghurt, probiotic drink and domestic curd samples of 11 g were weighed or pipetted individually and transferred to the sterile 99 ml flask containing phosphate buffer solution to make 1st dilution. Further required dilutions were prepared serially using 1st dilution. Required serial dilutions were transferred to the labelled sterile petri plates for the enumeration

of lactic cocci (that include *lactococci* of curd sample and *streptococcus thermophilusi* of yogurt) and lactobacilli using sterile pipettes. Respective sterile molten agar media such as M17, DeMann Rogosa Sharpe – MRS [readymade media purchased from HiMedia, Mumbai - RM reconstitution of dehydrated powder as well formulated media (FM) by weighing each ingredient and prepared the media] and Yeast glucose agar(YGA) [26], Elliker's agar(EA) [26], Selective YGA maintained at 50˚C of 10- 15ml were poured to labelled sterile petri plates, mixed gently and allowed to solidify. All M17 plates were incubated at 30˚C/24-48 h while MRS plates at and at 37˚C/24-48 h in candle jar. After the completion of the incubation period, the colonies were counted in countable plates ranging between 30-300 by colony counter and average count was expressed as cfu/g and further converted to log_{10}cfu/g of fermented milk product. The selected colonies of LAB from respective agar media from fermented milk products were used for microscopic observation and activity checking.

2.2 Incorporation of Inhibitors and pH Indicators to Media Used for Lactic Counts in Fermented Milk Samples

Inhibitors to control microbial contaminants like aerobic spore forming bacteria and yeast as well to differentiate lactic cocci and lactobacilli colonies pH indicators were incorporated to media used for lactic counts.

2.2.1 Incorporation of inhibitors to media used for obtaining lactic counts

Sterile solutions of calcium propionate (CP) to inhibit aerobic spore forming bacteria and sodium benzoate to control the growth of yeast were added to sterile molten to M17 at 0.50, 0.75, 1.0 and 1.5% while to MRS agar at 0.50, 0.75, 1.0,

1.5 and 2.0% individually and in combination, respectively for optimizing their levels. Sterile CP solution at 0.2, 0.4, 0.6 and 0.8 and sterile SB solution at 0.3, 0.6, 0.9 and 1.2 per cent were incorporated individually and in combination into sterile YGA.

2.2.2 Addition of pH indicator

Bromophenol blue (0.002 %) and bromocresol purple (0.005, 0.05, 0.5 %) were added to YGA and EA during media preparation, sterilized and then poured to the plates with dilutions of samples to differentiate the colonies of lactic cocci and lactobacilli.

2.3 Confirmation of Lactic Nature of Isolates Obtained from Media

The morphology of colonies that appeared on countable plates of YGA with inhibitors (CP- 0.8 +SB - 1.2) and pH indicator BCP (0.05) were noted. Both surface and submerged colonies were subjected for Gram's staining, catalase test and activity test in litmus milk, sterile skim milk.

2.3.1 Gram's staining

Smears of colonies were prepared on clean grease free slide, air dried treated with crystal violet solution for 1 min followed by Gram's iodine solution for 1 min, immediate gentle rinsing with water, decolorization with ethanol till colour goes off and final counter staining with safranine. Gram's staining helped in declaring the isolates as Gram positive or negative.

2.3.2 Activity test

The colonies selected were subjected to catalase, litmus milk tests (Harrigan, 1998) and titratable acidity in sterile skim milk (IS: SP: 18, 1981)

2.3.3 Catalase test

A small portion of colony selected was mixed with freshly prepared hydrogen peroxide (3 per cent). The presence of catalase was indicated by the liberation of free oxygen as gas bubbles by effervescence.

2.3.4 Litmus milk test

Sterile litmus milk was inoculated with small portion of colony selected, incubated at 30˚C for lactococci, 37˚C for *S.thermophilus* and lactobacilli for 24 h. Acid, reduction and coagulation was taken as positive for litmus milk reaction indicative of lactic nature of the colony.

2.3.5 Setting time and titratable acidity

The selected colonies were inoculated to sterile skim milk (9% total solids) incubated 30˚C for lactococci, 37˚C for *S.thermophilus* and lactobacilli for 18 h. The setting time of milk was noted. Acidity of the set milk was determined by taking exactly 10 g of well mixed sample in a conical flask. Phenolphthalein indicator of 5 drops was added and titrated against standardized 0.1 N sodium hydroxide (NaOH) and the rundown of NaOH was multiplied by 0.09 and expressed as percent Lactic acid.

2.4 Comparison of the Ready-Made Media and Formulated Media Used for LAB

The formulated media i.e., YGA with calcium propionate and sodium benzoate at 0.8 and 1.2 with BCP of 0.05 was compared with M17 and MRS counts obtained during enumeration of fermented milk products were correlated and validated through statistical analysis.

2.5 Statistical Analysis

Statistical analysis was carried out for the obtained data using R software (R-4.3.1 for Windows. The R Foundation for statistical Computing. https://cran.r-project.org/bin/windows/base/) to determine the significance of the treatments through the formula for the critical difference (CD).

$$CD = \frac{\sqrt{2} \times MSS(E)}{r} \times t\alpha \; at \; 0.05$$

where
MSS (E) = Mean Sum of squares of the error; r= number of replications; tα = t value at degrees of freedom at 0.05 probability. Same superscripts in the table column indicate non-significance while different superscripts indicate significance difference. Correlation coefficient (r) was calculated in Microsoft excel between selective YGA and M17 as well selective YGA and MRS agar counts.

3. RESULTS AND DISCUSSION

The results of enumeration obtained for lactic streptococci and lactobacilli in fermented milk products, using readymade media and formulated media are discussed in this section.

3.1 Enumeration of Lactic Cocci and Lactobacilli from Commercial and Domestic Fermented Dairy Products Using Formulated Media (FM) and Readymade Media (RM)

Fermented milk products such as market samples of curd, yogurt and domestic curd samples were enumerated. Treatments were performed using Formulated media (FM) and Readymade media (RM) like M17 for lactococci as well as *Streptococcus thermophilus* while MRS media was used for lactobacilli. The poured plates of lactococci and *Streptococcus thermophilus* as well as lactobacilli were incubated in candle jar at 30ºC and 37ºC, respectively for 48 h. One of the collected market yogurt sample showed high lactic acid of 1.02 per cent while one sample of domestic curd had lower acidity noticed of 0.68 per cent (Table 1). "The viable count of lactococci on formulated M17 medium in fermented milk samples ranged from 7.53 to 8.88 $\log_{10}$cfu/g (Table 1). The highest count was found in yogurt of about 8.88 $\log_{10}$cfu/g while market curd sample had low count of 7.53 $\log_{10}$cfu/g. But when readymade M17 medium was used, lactococci colonies ranged from 7.95 to 8.95 $\log_{10}$cfu/g in fermented products in which more viable count of 8.95 was observed in one brand of market yogurt sample and low viable count of 7.95 in one market curd sample" [27]. Viable count of lactobacilli was from 5.85 to 7.91 $\log_{10}$cfu/g in fermented products on formulated MRS agar. In readymade MRS agar, counts ranged from 6.00 to 7.96 $\log_{10}$cfu/g where highest count was found in one of the domestic curd sample of 7.96 $\log_{10}$cfu/g while in one of the market yogurts sample the viable count of lactobacill was 6.00 (Table 1).

The Readymade media used for pour plating gave better recovery of viable cells of lactic acid bacteria, from fermented milk samples due to use of pure forms of ingredients. The preparation of readymade media was easy as it was only reconstitution, no pH regulation followed by sterilization. Formulated media preparation on the other hand was time consuming as each ingredient to be weighed, dissolved in water, pH adjustment and then sterilization. Apart from that, recovery rate in formulated media was lower. Hence in the further studies readymade M17 and MRS were used. LAB is the most widely used starter culture in fermented foods, especially dairy products. LAB have the ability to adapt to different environments, which could explain their wide use in the fermentation of diverse food products. BRIGGS [5], a medium for streptococci and lactobacilli, LAE (Lactic-Agar-Elliker) [6], were developed. A number of lactobacilli strains did not grow well in any of these media, so a nonselective medium known as lactobacilli MRS [7] that was able to support the growth of lactobacilli was formulated. Many strains of LAB grow well at 42–43°C and give higher recoveries when enumerated using modified MRS media [28]. MRS and M17 were the most commonly used standard media, exhibiting consistent growth for lactobacilli and lactococci, respectively [29].

3.2 Effect of Incorporation of Calcium Propionate and Sodium Benzoate to Ready Made M17 and MRS Media on Lactic Counts of Fermented Milk Products

Market yogurt showed more acidity of 1.10 while domestic curd sample had lower acidity of 0.68 per cent, with range of 0.68 to 1.10 per cent lactic acid (Table 2) in fermented milk products. The viable count of lactic cocci in market curd, yogurt and domestic curd samples on control M17 agar was 8.98, 8.70, 8.80 that showed reduced to 8.60, 8.38, 8.48 (Table 2) on M17 with 1.5+1.5 per cents of calcium propionate and sodium benzoate both, which might be attributed to inhibition of aerobic spore formers as well as yeast.

Viable lactobacilli on MRS agar (control) were 8.30, 7.90, 8.88 and 8.53 $\log_{10}$cfu/g (Table 3) noticed in market curd, yogurt and domestic curd samples, respectively (Table 3).

But after incorporation of 2.0+2.0 per cent of calcium propionate and sodium benzoate, the counts were 7.80, 7.50 and 8.00 in market curd, yogurt and domestic curd samples, respectively. Reduction in counts was noticed in both M17 and MRS agar after incorporation of calcium propionate and sodium benzoate at 1.5 percent, and 2 percent, respectively. Such treatment helped in control of both aerobic spore formers and yeasts that appeared as surface colonies on both the media. The effect of the inhibitors on microbial contaminants might be ascribed to leakage of proton motive force that affected ATP synthesis of both contaminants that in turn led to death due to starvation [23,24]. Thus, incorporation of calcium propionate and sodium benzoate could be successfully practiced in the laboratories for the microbiological analysis of fermented milk products to get actual counts of lactic cocci and lactobacilli.

Table 1 Enumeration of lactic acid bacteria from commercial and domestic fermented dairy products using Formulated media (FM) and Readymade (RM)

Sample name	Code	Titratable Acidity	Viable count			
			Lactococci/ *Str. thermophilus* (in curd/yogurt)		Lactobacilli (in curd/ yogurt)	
			M17		MRS	
			FM	RM	FM	RM
		% Lactic acid	Viable count (log_{10}cfu/g)			
Commercial fermented dairy products samples						
Curd	MC1	0.76 [a]	7.59 [a]	8.00 [a]	7.17 [a]	7.60 [a]
	MC2	0.80[a]	7.56 [a]	7.95 [a]	7.57 [a]	7.77 [a]
	MC3	0.81[a]	7.53 [a]	8.00 [a]	6.87 [a]	7.00 [a]
ogurt	MY1	1.02[a]	8.88 [a]	8.95 [a]	5.85 [a]	6.00 [a]
	MY2	1.00 [a]	8.80 [a]	8.91 [a]	7.00 [a]	**7.**11 [a]
Domestic samples						
Curd	HC1	0.68[a]	8.68 [a]	8.88 [a]	7.69 [a]	7.89 [a]
	HC2	0.72[a]	8.65 [a]	8.90 [a]	7.76 [a]	7.96 [a]
	HC3	0.83[a]	8.50 [a]	8.78 [a]	7.91 [a]	7.67 [a]
CD (*P*=.05)		**2.53**	**2.61**	**2.59**	**2.30**	**2.45**

- *CD, Critical difference; all the values are average of three trials; same superscripts in the column indicate non-significance; different superscripts indicate significance difference*

Table 2. Effect of incorporation of calcium propionate and sodium benzoate incorporated to M17 and MRS on the lactic counts of fermented milk products

Name of the sample	Sample code	Titratable Acidity (% LA)	Control M17	Control MRS	Addition of calcium propionate + sodium benzoate %								
					0.50 + 0.50	0.75 + 0.75	1.0 + 1.0	1.5 + 1.5	0.50 + 0.50	0.75 + 0.75	1.0 + 1.0	1.5 + 1.5	2.0 + 2.0
			M17		MRS								
			Viable count (log_{10}cfu/g)										
Market curd	MC1	0.72[a]	8.98[a]	8.30[a]	8.90[a]	8.86[a]	8.80[a]	8.60[a]	8.00[a]	7.95[a]	7.90[a]	7.84[a]	7.80[a]
Market yogurt	MY1	1.10[a]	8.70[a]	7.90[a]	8.62[a]	8.58[a]	8.46[a]	8.38[a]	7.84[a]	7.78[a]	7.70[a]	7.60[a]	7.50[a]
Domestic curd	HC1	0.68[a]	8.80[a]	8.53[a]	8.68[a]	8.60[a]	8.51[a]	8.48[a]	8.43[a]	8.36[a]	8.20[a]	8.02[a]	8.00[a]
CD (*P*=.05)		**1.21**	**1.23**	**1.15**	**1.23**	**1.23**	**1.23**	**1.23**	**1.08**	**1.08**	**1.07**	**1.10**	**1.11**

Table 3. Effect of incorporation of optimized calcium propionate and sodium benzoate to M17 and MRS media on lactic counts of fermented milk products

Sample name	Sample code	Medium used for plating lactic cocci and lactobacilli			
		M17 control	MRS control	M17 with 1.5 % calcium propionate and 1.5 % sodium benzoate	MRS with 2 % calcium propionate and 2 % sodium benzoate
		log_{10}cfu/g			
Market curd	MC1	8.60[a]	8.00[a]	8.34[a]	7.80[a]
	MC2	8.94[a]	8.58[a]	8.56[a]	8.34[a]
	MC3	8.96[a]	8.30[a]	8.60[a]	8.00[a]
Market yogurt	MY1	8.70[a]	5.86[a]	8.44[a]	5.66[a]
	MY2	8.58a	8.10[a]	8.32[a]	7.86[a]
Domestic curd	HC1	8.56[a]	8.80[a]	8.46[a]	8.60[a]
	HC2	8.59[a]	8.46[a]	8.40[a]	8.26[a]
	HC3	8.53[a]	8.80[a]	8.31[a]	8.62[a]
CD (*P*=.05)		**4.43**	**4.27**	**4.43**	**4.30**

Table 4. Enumeration of lactococci and lactobacilli from curd sample using Yeast Glucose Agar (YGA) and Elliker Agar (EA) with Bromocresol purple (BCP) and Bromophenol blue (BPB)

Sample name	Medium used for plating lactococci and lactobacilli					
	YGA	EA	Incorporation of BCP at 0.005 % to		Incorporation of BPB at 0.002 % to	
			YGA	EA	YGA	EA
	Viable count (log_{10}cfu/g)					
Market curd (MC1)	8.38[a]	8.16[a]	8.30[a]	8.00[a]	8.10[a]	7.80[a]
Domestic curd (HC1)	8.42[a]	7.84[b]	8.30[a]	7.70[a]	8.00[a]	7.50[b]
CD (*P*=.05)	**0.57**	**0.24**	**0.73**	**0.74**	**0.48**	**0.17**

3.3 Enumeration of Lactococci and Lactobacilli from curd Samples Using Yeast Glucose Agar and Elliker's Agar with pH Indicators

In order to formulate a single medium for both lactococci and lactobacilli only curd samples obtained from market and home were tried. After optimization the standardized medium was used to obtain LAB present in other fermented milk products. In order to enumerate LAB present in market and domestic curd samples, yeast glucose agar and Elliker's agar were used as such and also incorporated with pH indicators bromocresol purple (0.005 per cent) and bromophenol blue (0.002 per cent). Yeast glucose agar and Elliker agar media considered as general-purpose media for lactic acid bacteria when used for plating of market and domestic curd samples, had viable counts of 8.38 and 8.42 (Table 4) while Elliker's agar expressed lesser counts of total lactic acid bacteria of 8.16 and 7.84 $\log_{10}$cfu/g, respectively. Similar mode was noticed in Bromocresol purple at 0.005 per cent and Bromophenol blue of 0.002 per cent incorporated yeast glucose agar and Elliker's agar separately, where Elliker agar with bromophenol blue failed to recover lactic acid bacteria to fuller extent when compared with yeast glucose agar with boromocresol purple as the counts obtained on Elliker agar was comparably lesser (Table 4).

On the contrary, Bromophenol blue (BPB-0.002 per cent) instead of Bromocresol purple (BCP-0.005 per cent) was recommended in MRS agar when compared with Plate count agar as MRS-BPB that led to maximum recovery of probiotic cultures in yogurt drink of $1x10^8$ compared to PCA-BCP enumeration of LAB ($2.5x10^7$) with blue-coloured lactobacilli colonies and it allowed differentiation of each LAB in a mixed culture [15].

3.3.1 Enumeration of lactococci and lactobacilli from curd samples using yeast glucose agar and Elliker agar with pH indicators compared with direct microscopic count

Yeast glucose agar and Elliker's agar were used as such and also incorporated with pH indicators bromocresol purple (0.005 per cent) and bromophenol blue (0.002 per cent). The direct microscopic counts of both curd samples were around 8 log count for cocci, rods and yeasts of market and domestic curd samples respectively (Table 5).

The viable counts on yeast glucose agar were better and nearly same as DMC but Elliker agar showed lower counts of LAB when market and domestic curd samples were plated. Bromocresol purple was added at 0.005 per cent to yeast glucose agar, market and domestic curd samples were plated three types of colonies obtained were surface, yellow & purple colonies in market and domestic curd samples. Bromophenol blue of 0.002 per cent in yeast glucose agar and Elliker agar when used for plating did not show colony differentiation with respect to colour, Hence, use of yeast glucose agar and Elliker agar with boromophenol blue was stopped in further studies. Yeast glucose agar with Bromocresol Purple (BCP) was used which showed better viable counts of lactic acid bacteria than Elliker agar with BCP. The two pH indicators bromocresol purple and bromophenol blue, bromocresol purple were compared bromocresol purple worked better with colony differentiation regard to colours like yellow and purple. A medium for streptococci and lactobacilli, LAE (Lactic-Agar-Elliker), was developed [6] that contained tryptone, yeast extract, gelatin, glucose, sucrose, lactose sodium chloride, sodium acetate, ascorbic acid with pH 6.8. While 50 mg/ml of 2,3,5- triphenyltetrazolium chloride with 1 per cent Tween 80 supplemented to Elliker's lactic agar helped to produce small, red *Streptococcus thermophilus* colonies and larger, white *Lactobacillus bulgaricus* colonies. Addition of 7 per cent skim milk (11 per cent solids) to lactic agar in place of 2,3,5-triphenyltetrazolium chloride allowed good rod-coccus differentiation, regardless of strain or yogurt brand. On this medium, called yogurt lactic agar, *L. bulgaricus* appeared as large white colonies surrounded by a cloudy zone and *S. thermophilu*s as smaller white colonies devoid of a surrounding halo [13]. Yogurt lactic agar compared favorably with *S. thermophilus* and Lactobacillus agar media for the recovery of *S. thermophilus* and *L. bulgaricus* in single and mixed culture. A total of 10 sucuk (Turkish-type fermented sausage) samples, obtained from Denizli, had on an average of 8.34 log CFU/g TAMB, 8.91 log CFU/g LAB (at the MRS agar) and average 8.25 log CFU/g LAB (at the Elliker's lactic agar) both lactobacilli and lactic acid producing cocci [14]. Even HiMedia [30] also had developed and recommended for cultivation of lactobacilli and streptococci of importance in dairy industry.

Table 5. Enumeration of lactococci and lactobacilli from curd sample using Yeast Glucose Agar (YGA) and Elliker Agar (EA) with optimized levels of Bromocresol purple (BCP) and Bromophenol blue (BPB) compared with direct microscopic count

Sample name	Type of cells			Medium used for plating lactococci and lactobacilli							
	Cocci	Rods	Yeast	YGA	EA	Incorporation of BCP at 0.005 %			EA	Incorporation of BPB at 0.002 %	
						YGA				YGA	EA
						Colony type					
						S	Y	P			
	DMC(log_{10}cells/g)			Viable count (log_{10}cfu/g)							
Market curd (MC1)	8.69[a]	8.48[a]	7.98[a]	8.38[a]	8.16[a]	7.88[a]	8.30[a]	8.60[a]	8.00[a]	8.10[a]	7.80[a]
Domestic curd (HC1)	8.98[b]	8.52[a]	7.80[a]	8.42[a]	7.84[b]	7.69[a]	8.42[a]	8.80[a]	7.70[a]	8.00[a]	7.50[b]
CD (*P*=.05)	**0.24**	**0.62**	**0.36**	**0.58**	**0.24**	**0.24**	**0.44**	**0.27**	**0.47**	**0.73**	**0.24**

Note: S – surface colony; Y – Yellow colony; P – Purple colony

Table 6. Optimization of addition of bromocresol purple (BCP) to yeast glucose agar for the enumeration of lactic colonies in curd sample

Name of the sample	Yeast glucose agar with BCP (%)								
	0.005			0.05			0.5		
	Type of colony								
	S	Y	P	S	Y	P	S	Y	P
	Viable count (log_{10}cfu/g)								
Market curd (MC1)	7.88[a]	8.30[a]	8.60[a]	7.70[a]	8.32[a]	8.56[a]	7.50[a]	8.38[a]	8.58[a]
Domestic curd (HC1)	7.69[a]	8.42[a]	8.80[a]	7.60[a]	8.44[a]	8.78[a]	7.40[a]	8.40[a]	8.72[a]
CD (*P*=.05)	**0.30**	**0.51**	**0.44**	**0.51**	**0.48**	**0.32**	**0.47**	**0.64**	**0.38**

Note: S – surface colony; Y – Yellow colony; P – Purple colony

3.3.2 Effect of addition of bromocresol purple (BCP) to yeast glucose agar on the differentiation of colonies of lactococci and lactobacilli

Bromcresol purple is a pH indicator that differentiated the colonies of lactic cocci and bacilli present in fermented milk products. With this background, 0.005, 0.05 and 0.5 per cent of BCP was added to yeast glucose agar and poured to plated market and domestic curd samples (Table 6).

"Viable counts of surface, yellow and purple colonies on yeast glucose agar containing BCP of 0.005, 0.05 and 0.5 per cents did not show much difference, BCP added at 0.005 per cent faded the colour of colonies on 2nd day and became difficult to take the counts while 0.05 per cent the colour of the colonies remained the same throughout the incubation period. BCP with 0.5 per cent led to dark colouration and again the colonies were difficult to count. Yeast glucose agar with 0.05 per cent bromocresol purple was used to obtain viable counts of lactic acid bacteria in further studies" [27].

3.3.3 Optimization of medium for the enumeration of mixed flora of lactococci and lactobacilli from curd samples using yeast glucose agar with BCP, calcium propionate and sodium benzoate:

Addition of calcium propionate at 0.2, 0.4, 0.6 and 0.8 per cents to yeast glucose agar with 0.05 per cent BCP when used for lactic counts in market and domestic curd samples, the surface colonies means aerobic spore forming bacterial colonies reduced to 0.00 while counts of both yellow indicated lactobacilli and purple colonies indicated lactococci were more than 8.50 $\log_{10}$cfu/g (Table 7).

Sodium benzoate when added at 0.3, 0.6, 0.9 and 1.2 per cents to yeast glucose agar with 0.05 per cent BCP same trend was noticed with respect to surface colonies that drastically came to nil whereas yellow colonies (lactobacilli) and purple colonies (lactococci) increased in their numbers. Overall reduction of surface colonies and increase in lactic acid bacterial colonies were observed when 0.8 per cent calcium propionate that inhibited spore forming bacteria and 1.2 per cent sodium benzoate inhibited yeast incorporated in yeast glucose agar containing 0.05 per cent BCP.

3.4 Use of Selective Yeast Glucose Agar with 0.05 Per Cent BCP and Optimized Levels of Calcium Propionate as Well as Sodium Benzoate for the Enumeration of Lactic Cocci and Lactobacilli in Fermented MILK PRODucts

Market samples of curd, yogurt as well domestic curd were subjected for enumeration of lactic acid bacteria present, selective yeast glucose agar was used that contained yeast glucose agar with 0.05 per cent of BCP, calcium propionate of 0.8 per cent and sodium benzoate of 1.2 per cent. Market curd (MC1) and yogurt (MY1) samples showed higher lactic count of more than 8 log (8.84, 8.85 $\log_{10}$cfu/g), yellow colonies that indicated lactobaciili (through microscopic examination) of 8.16 log count on an average while purple colonies of lactococci of 8.51 $\log_{10}$cfu/g were noticed in higher total lactic counts among fermented milk products. Two market curd (MC2, MC3), domestic curd (HC1, HC3) as well market yogurt (MY2) samples exhibited nearly 7.72 log on an average of total lactic count on yeast glucose agar with 0.05 per cent BCP, yellow colonies of lactobacilli of 7.20 log and nearly same counts of purple colonies were noticed (Table 8).

Thus, yeast glucose agar with BCP of 0.05 per cent + calcium propionate of 0.8 per cent and sodium benzoate of 1.2 per cent could be used as selective media for total lactic count in fermented milk products that also differentiates yellow colony as lactobacilli a nd purple colony for lactic cocci, an added advantage of the medium. On par with the present study, 50 mg/ml of 2,3,5-triphenyltetrazolium chloride, a redox indicator was added to Elliker's lactic agar to produce small, red *Streptococcus thermophilus* colonies and larger, white *Lactobacillus bulgaricus* colonies from commercial yogurt samples [13]. On the contrary MRS with Bromo phenol blue showed advantages in enumeration of LAB due to incubation time than Plate count agar with Bromocresol purple, that allowed differentiation of each LAB in a mixed culture [15]. M1 agar, containing two chromogenic substrates (β-galactosidase & β-glucosidase), allowed selective enumeration *of Lactobacillus rhamnosus*, two strains of *Lactobacillus*

paracasei ssp. *paracasei* and *Streptococcus thermophilus* based on differential β - galactosidase and β -glucosidase activities and incubation at 37 °C or 44 °C to increase selectivity. A second agar medium, M2, containing one chromogenic substrate (β-galactosidase) was used to selectively enumerate β-galactosidase producing *Lactobacillus delbrueckii* ssp. *Bulgaricus* at 47 °C. In contrast, with the usual culture media, the chromogenic method allowed unambiguous enumeration of each species, including discrimination between the two *L. paracasei*, up to 10^9 CFU/g of fermented milk [31].

3.5 Activity of Lactic Colonies Appeared on Selective Yeast Glucose Agar

The yellow submerged colonies that appeared on yeast glucose agar containing 0.05 per cent BCP with 0.8 per cent calcium propionate and 1.2 per cent sodium benzoate obtained from curd sample microscopically revealed rods when inoculated to sterile skim milk set the curd in 8 h had 0.63 to 0.68 per cent lactic acid (Table 9). Purple colonies that appeared on selective yeast glucose agar when curd or yogurt was plated demonstrated as Gram positive cocci through Gram's staining with titratable acidity of 0.63 to 0.66 per cent lactic acid that set the milk in 8 h after inoculation of colony. Both the colonies revealed catalase positive and acid, reduction and coagulation in litmus milk.

The confirmation of Gram positive rods and cocci indicated lactobacilli and lactic cocci that appeared on yeast glucose agar with 0.05 per cent BCP + 0.8 per cent calcium propionate and 1.2 per cent sodium benzoate made it as a prime medium for fermented milk products to get total lactic counts instead of using separate media for lactic cocci (M17 agar) and lactobacilli (MRS agar). LAB strains (13) were identified on the basis of phenotype out of which seven isolates (53.8 per cent) were found to belong to the genus Lactobacillus while the remaining six isolates (46.2 per cent) were under the genus Streptococcus. Identified Lactobacillus species included *L. delbrueckii, L. hilgardii*, and *L. plantarum* while *Streptococcus* species were identified as S*. faecalis, S. lactis, S. thermophilus* and *S. faecium*. Among the identified all isolates, *S. lactis, L. delbrueckii* produced the highest acid (w/v) 0.46 per cent after 72 h of incubation period. The isolates were obtained from the yoghurt samples with higher counts of lactic acid bacteria (LAB) that ranged from 1.0×10^6 to 5.6×10^7 cfu/mL and 2.2×10^7 to 5.4×10^8 cfu/mL on MRS agar (pH 6.2-6.6) and Rogosa agar (pH 5.2- 5.6) for total LAB enumeration as well as lactobacilli isolation while YGLA (pH 7.0) for the isolation of streptococci incubated at 37°C for 48-72 h at anaerobic conditions [19]. On par with the present study, the formulated selective Yeast glucose agar with 0.8 and 1.2 per cents of calcium propionate and sodium benzoate, respectively to control aerobic spores and yeast (common contaminants of fermented milk products) along with bromocresol purple of 0.05 per cent helped to form purple coloured colonies of for lactic cocci from market curd, yogurt and domestic curd samples [32]. Even plate count agar with BCP showed better counts of lactobacilli in fermented milk samples [33].

3.6 Comparison of Lactic Counts of Fermented Milk Products on Selective YGA

Comparison of viable counts on M17 and MRS with calcium propionate and sodium benzoate with selective yeast glucose agar containing bromocresol purple with calcium propionate and sodium benzoate was carried out. Selective M17 (1.5 % calcium propionate + 1.5 % sodium benzoate) and selective YGA (BCP 0.05 % 0.8 calcium propionate + sodium benzoate 1.2 % - purple colonies as lactococci. had around 8 log viable count of lactococci in fermented milk products. Selective MRS and YGA (yellow colonies as lactobacilli) media also showed similar counts of lactobacilli. The correlation coefficient, r was 0.78 between selective M17 of lactococci and selective yeast glucose agar for purple colony of lactic cocci (this might include lactococci, streptococci, leuconostoc or pediococci) while r was 0.67 (Table 10) when MRS lactobacilli count was compared with yellow colonies of lactobacilli on selective yeast glucose agar indicating good relationship between both the types of selective agar media and better relationship was between selective M17 agar and selective (differential) yeast glucose agar with respect to purple colonies of lactic cocci.

Table 7. Enumeration of lactococci and lactobacilli from curd sample using Yeast Glucose Agar (YGA) with Bromocresol purple (BCP), calcium propionate and sodium benzoate

Sample name	Medium used for plating lactic acid bacteria									
	YGA + 0.05 % BCP	Colony Type	YGA + 0.05 % BCP							
			Calcium propionate (%)				Sodium benzoate (%)			
			0.2	0.4	0.6	0.8	0.3	0.6	0.9	1.2
			Viable count (log_{10}cfu/g)							
Market curd (MC1)	8.48^{a}	S	7.20^{a}	5.02^{a}	3.26^{a}	0.00^{a}	7.10^{a}	5.00^{a}	3.00^{a}	0.00^{a}
		Y	8.60^{a}	8.70^{b}	8.78^{b}	8.88^{b}	8.40^{a}	8.60^{b}	8.70^{b}	8.80^{b}
		P	8.80^{b}	8.88^{c}	8.92^{ca}	8.98^{ca}	8.80^{a}	8.84^{ca}	8.90^{ca}	8.98^{ca}
Domestic curd (HC1)	8.65^{a}	S	7.90^{a}	6.80^{d}	3.62^{ab}	0.00^{ab}	7.60^{a}	5.40^{ab}	3.20^{ab}	0.00^{ab}
		Y	8.62^{a}	8.45^{e}	8.82^{da}	8.62^{da}	8.60^{a}	8.76^{da}	8.86^{da}	8.98^{da}
		P	8.72^{a}	8.80^{f}	8.88^{ea}	8.92^{ea}	8.78^{b}	8.90^{ea}	8.96^{ea}	9.00^{ea}
CD (*P*=.05)	**0.27**		**1.55**	**1.56**	**1.69**	**1.71**	**1.51**	**1.65**	**1.69**	**1.70**

Note: *S – surface colony; Y – Yellow colony; P – Purple colony*

Table 8. Enumeration of lactic cocci and lactobacilli from fermented dairy products using selective Yeast Glucose Agar (YGA) with optimized levels of Bromocresol purple (BCP) with both calcium propionate and sodium benzoate

Sample name	Sample code	Medium used for plating lactic acid bacteria		
		YGA with 0.05 % Bromocresol purple (control)	YGA + 0.05 % BCP + 0.8 % Calcium propionate +1.2 % Sodium benzoate (selective YGA)	
			Colony type	
			Yellow	Purple
		Viable count (log_{10}cfu/g)		
Market curd	MC1	8.84^{a}	8.16^{a}	8.56^{a}
	MC2	7.77^{a}	7.30^{a}	6.95^{a}
	MC3	7.60^{a}	7.00^{a}	6.95^{a}
Market yogurt	MY1	8.85^{a}	8.00^{a}	7.27^{a}
	MY2	7.47^{a}	8.47^{a}	7.32^{a}
Domestic curd	HC1	7.90^{a}	7.20^{a}	7.50^{a}
	HC2	8.50^{a}	8.20^{a}	8.48^{a}
	HC3	7.60^{a}	7.27^{a}	7.47^{a}
CD (*P*=.05)		**2.54**	**2.59**	**2.57**

Table 9. Activity of lactic colonies appeared on selective yeast glucose agar

Name of the sample	Selective yeast glucose agar (YGA + 0.05 % BCP + 0.8 % Calcium propionate +1.2 % Sodium benzoate)			
	Colony morphology	Cell morphology	Setting Time(h)	Titratable Acidity (% Lactic acid)
Market curd (MC1) /Domestic curd ((HC1)	Yellow submerged	Rods	8	0.63[a]
	Purple submerged	Cocci	8	0.66[a]
Market yogurt (MC1)	Yellow submerged	Rods	8	0.68[a]
	Purple submerged	Cocci	8	0.63[a]
CD (*P*=.05)				**1.78**

Table 10. Comparison of lactic counts of fermented milk products on selective yeast glucose agar

Sample name	Sample code	Medium used for plating lactic acid bacteria			
		M17+ 1.5 % Calcium Propionate (CP) + 1.5 % Sodium Benzoate (SB) (lactic cocci)	YGA+ BCP 0.05% + CP 0.8%+ SB 1.2% (selective YGA) Purple colonies(lactic cocci)	MRS+ CP 2 % + SB 2 % (lactobacilli)	YGA+BCP 0.05 % + CP 0.8 % + SB 1.2 % (selective YGA) Yellow colonies (lactobacilli)
		log_{10}cfu/g			
Market curd	MC1	8.10	8.26	8.00	8.16
Market yogurt	MY1	8.08	8.10	7.80	8.00
Domestic curd	HC1	8.18	8.20	8.00	8.20
r (correlation coefficient)		**0.78**		**0.67**	

4. CONCLUSION

The readymade medium for lactic streptococci M17 agar medium gave better results when compared to formulated medium. The formulated medium with inhibitory agents like calcium propionate (0.8 %) and sodium benzoate (1.2 %) helped to curb the common contaminants like aerobic bacterial spore formers and yeast, respectively in the fermented milk products. Instead of using two selective media such as M17 and MRS agar media for lactic cocci and lactobacilii, respectively, in this study an attempt was made to convert general purpose medium yeast glucose agar (YGA) to selective and differential medium for both the microflora which can be identified by colony colour differentiation. The calcium propionate and sodium benzoate along with pH indicator bromocrseol purple incorporation to yeast glucose agar medium, a general purpose medium can be easily converted to differential medium. The viable lactic cocci formed purple colonies while yellow colonies of lactobacilli as a measure of differentiation based on acid production in case of mixed lactic flora in fermented milk products. The inhibitors calcium propionate and sodium benzoate incorporated in the YGA inhibited the most common microbial contaminants in fermented milk products, aerobic spore formers and yeasts, respectively.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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