ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Analysis of Important Dairy Products: Isolation and Characterization

LK Attri* and Humeet Narang

Desh Bhagat University, Mandi Gobindgarh-147 301, Punjab, India.

ABSTRACT

In the present study, different daily use dairy products were procured and isolation was carried out using standard morphological. Based upon the morphological and biochemical standard test, the isolates were confirmed to be *E.coli, Pseudomonas, Lactobacillus* and *Staphylococcus*. Different biochemical standard methods including Indole production test, Methyl-Red and Voges-Proskauer (MRVP) test, Catalase Test and Citrate Utilization Test etc. were performed for final conclusion. Therefore, the results showed the presence of harmful bacterias in daily use dairy product. Further, Slants of nutrient agar media were made and kept for solidification. After solidification pure colonies of isolates were streaked on these slants and incubated at 37° C for 2-3 days. After growth these slants were stored at 4° C for further use.

Keywords: Antibacterial agents, bacterias, standard tests, nutrient medias

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Dairy products are generally defined as food produced from the milk of mammals (the Food Standards Agency of the United Kingdom defines *dairy* as "foodstuffs made from mammalian milk). They are usually high energy-yielding food products. A production plant for the processing of milk is called a dairy or a dairy factory. Apart from breastfed infants, the human consumption of dairy products is sourced primarily from the milk of cows, goats, sheep, yaks, camels, and other mammals are other sources of dairy products consumed by humans. Dairy products are commonly found in European, Middle Eastern and Indian cuisine, whereas they are almost unknown in East Asian cuisine.

Milk, an extremely nutritious food and is a major part of human food and diet, is an aqueous colloidal suspension of proteins, fats and carbohydrates that contains numerous vitamins and minerals. Due to its complex biochemical composition and high water activity, milk serves an excellent culture media for growth of many kinds of microorganism; some of microorganisms produce undesirable effects to consumers.

Several milk products including cultured dairy products (such as cheese, paneer, curd yogurt, kefir and clabbered milk), known as dairy products are available in the market for their use and have been inseparable part of the human diet since time immemorial; these are all forms of cultured, soured or fermented milk. On the other hand, fermented milk products are not consumed directly but often form starting materials from which other dairy products are also manufactured [1,2].

In India, different fermented products are prepared from milk. These products are mostly intended primarily to conserve the nutritional values of milk [3]. The recognition of dairy products with probiotics bacteria as functional foods that provide health benefits beyond basic nutrition and emerging clinical evidence to their potential in preventing some diseases have notable enlarged their consumption and stimulated innovation and new product development [4,5].

Despite of its immense potential benefits, unfortunately, it also serves as an important medium for transmission of pathogenic organisms and as observed that fresh, non pasteurized milk generally contains varying numbers of microorganisms based upon its care employed in milking, cleaning, and handling of milk utensils.

Incidentally, in India subcontinent, such products, called Indigenous milk products are major source of calcium, magnesium and phosphorous, also carry toxic metabolites of organisms and ingestion of these metabolites cause food borne infections. It has been observed, invariably that soft cheeses (e.g., brie, cheese, cottage cheese) having more water content prepare a suitable environment for bacteria, viruses or molds to multiply quickly [6 ,7].
With a view to the above literature, a study has been planned (a) to analysis of variety of daily use dairy products and isolation of bacteria and (b) To identify and characterize the isolated strains based on morphological and biochemical tests.

ISSN: 0975-8585

MATERIALS AND METHODS

Collection of samples

The present study, as many as 10 dairy samples were collected from different sites/ locations. Each sample was labelled to show serial number and source. The samples were collected in sterilized screw capped containers and transported to lab. All samples were kept at 4^0C until processed. The type of dairy sample, source and sample number is given in table 1.

Table 1: Showing type of sample, sample number and source.

S.NO	SAMPLE	SAMPLE NO.	SOURCE
1.	Paneer	1.	Dairy
2.	Khoya	2.	Dairy
3.	Curd	3.	Surya
4.	Butter	4.	Vimal
5.	Milk	5.	Verka
6.	Mayonnaise	6.	Mr. Bactor
7.	Lassi	7.	Verka
8.	Cream	8.	Amul
9.	Icecream	9.	Basant
10.	Flavoured milk	10.	Nestle

Processing of samples

Isolation of various microorganisms from different samples

Serial dilution

1 ml of the sample was mixed in 9 ml sterile normal saline (0.85% Nacl) test tube. 9 test tubes were taken for each sample and were labelled as 1 to 9. Uniform suspension was made by vigorous shaking on magnetic shaker for few min. The resultant suspension was called master sample. Master sample is further serially diluted 10 folds from 10^{-1} to 10-9 concentration. 15 ml of selective media was poured in each petridish and allowed to solidify. Upon solidification of media, 0.1 ml of inoculum was spreaded into petriplates with the help of spreader under aseptic conditions. Procedure was repeated for all other samples and labelled. All plates were incubated at their respective temperatures for 24- 48 h in an incubator in inverted form and were observed for appearance of distinct individual colonies.

E.Coli was isolated on Eosin Methylene Blue (Emb) Agar and Macconkey Agar Plates Whereas *Lactobacillus* was isolated using De Man Ragossa Sharpe (Mrs) Medium; Kings Medium was used for isolation of *Pseudomonas* while *Staphylococcus* was isolated by using Baird Parker Agar and Blood Agar**.**

ISSN: 0975-8585

The isolated colonies were mixed with many forms of life therefore, the sample obtained was inoculated on petriplates in order to obtain pure culture. The isolates so obtained were identified using colony morphology, gram's staining methods and Biochemical tests.

The morphological and biochemical characterizations were carried out using gram staining by standard methods [8] and for biochemical identification of bacterias, different standarderized test have been used including Indole production test, Methyl-Red and Voges-Proskauer (MRVP) test, Catalase Test and Citrate Utilization Test.

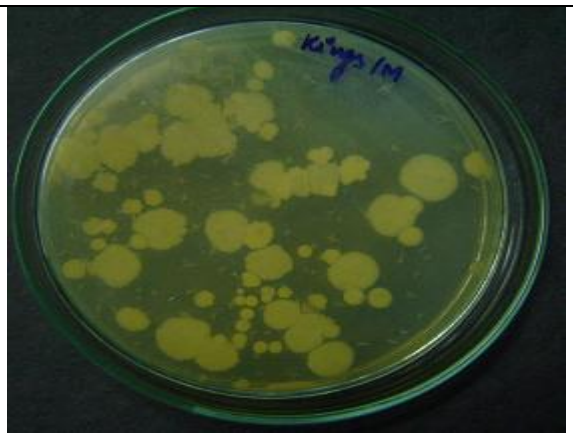
Further, Slants of nutrient agar media were made and kept for solidification. After solidification pure colonies of isolates were streaked on these slants and incubated at 37° C for 2-3 days. After growth these slants were stored at 4° C for further use.

RESULTS AND DISCUSSION

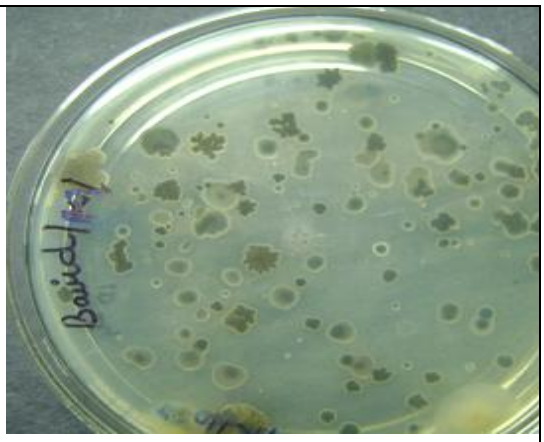
In the present study, *E.coli, Pseudomonas*, *Lactobacillus, Staphylococcus* were isolated from different dairy products. Isolates were confirmed by morphological and biochemical procedures. Five biochemical tests widely employed in the classification of *E.coli, Pseudomonas, Lactobacillus, Staphylococcus* were done to confirm their presence.

Isolation analysis of dairy products

Bacteriological examination of dairy samples was done to check the contamination in samples. For this, serial dilution was done and then samples was streaked on different culture medias (Figure 1) and the results obtained has been given below (Table 2).



a



b

ISSN: 0975-8585

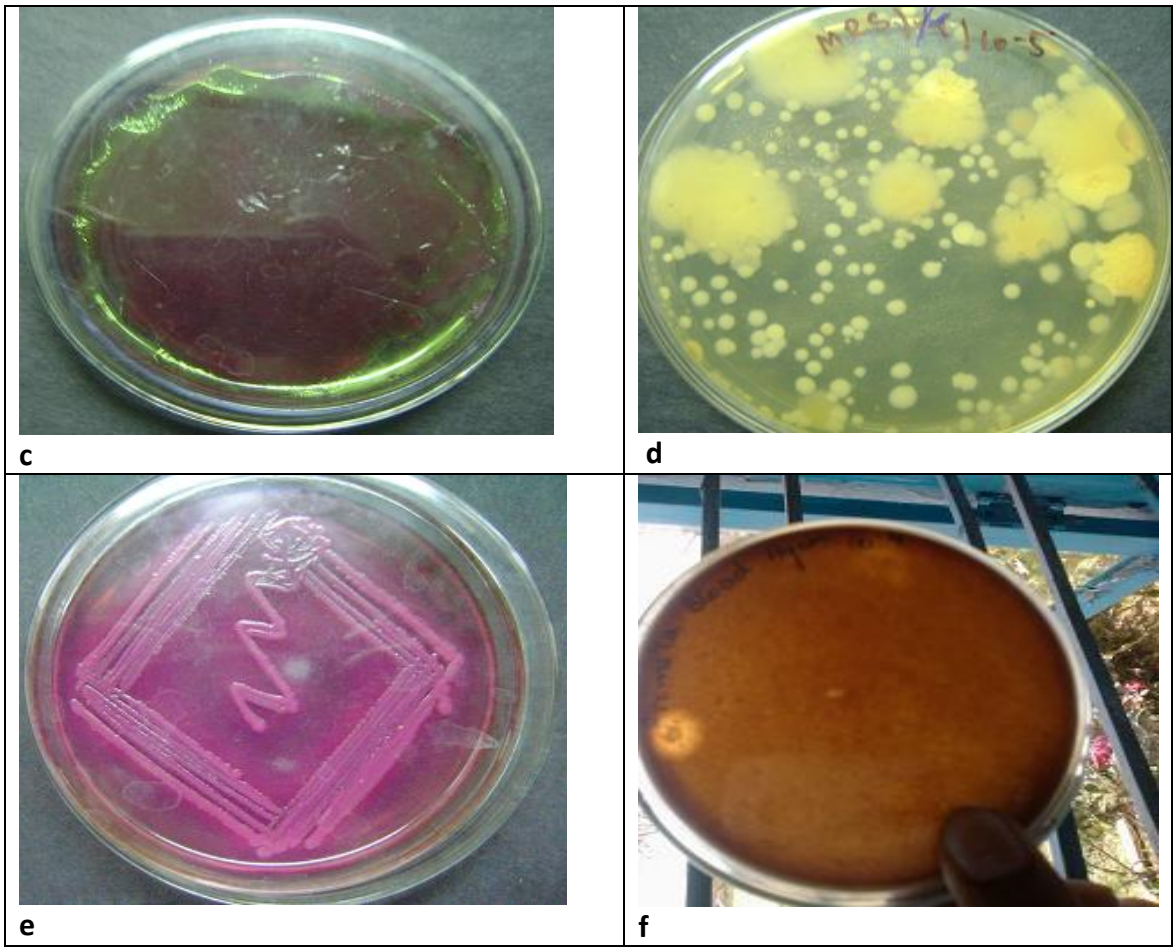


Fig-1: Showing isolates from various dairy samples; a) *Pseudomonas;* b) *Staphylococcus;* c) *Escherichia coli;* d) *Lactobacillus;* e) *Escherichia coli* on MacConkey agar; f) *Staphylococcus* on Blood agar.

Table 2. Bacteriological analysis of dairy samples from different sources.

S.No	SAMPLE	*E.coli*	*Pseudomonas*	*Lactobacillus*	*Staphylococcus*
1.	Paneer	_	+	+	+
2.	Khoya	_	+	+	+
3.	Curd	+	+	+	+
4.	Butter	_	+	+	+
5.	Milk	_	+	+	+
6.	Mayonnaise	+	+	+	+
7.	Lassi	_	+	+	_
8.	Cream	+	+	+	+
9.	icecream	_	_	_	_
10.	Flavoured milk	_	_	_	_

Based upon morphological characteristics by observing features (Table 3), four bacterias has been recognized (Figure 2).

ISSN: 0975-8585

Table 3: Morphological Characterization of Isolates namely *E.coli, Pseudomonas, Staphylococcus* and *Lactobacillus* [9]

Name of Isolates	Characters
Escherichia coli	Gram- negative, rod shaped
Pseudomonas	Gram-negative, rod-shaped
Lactobacillus	Gram-positive, rod shaped
Staphylococcus	Gram- positive, spherical

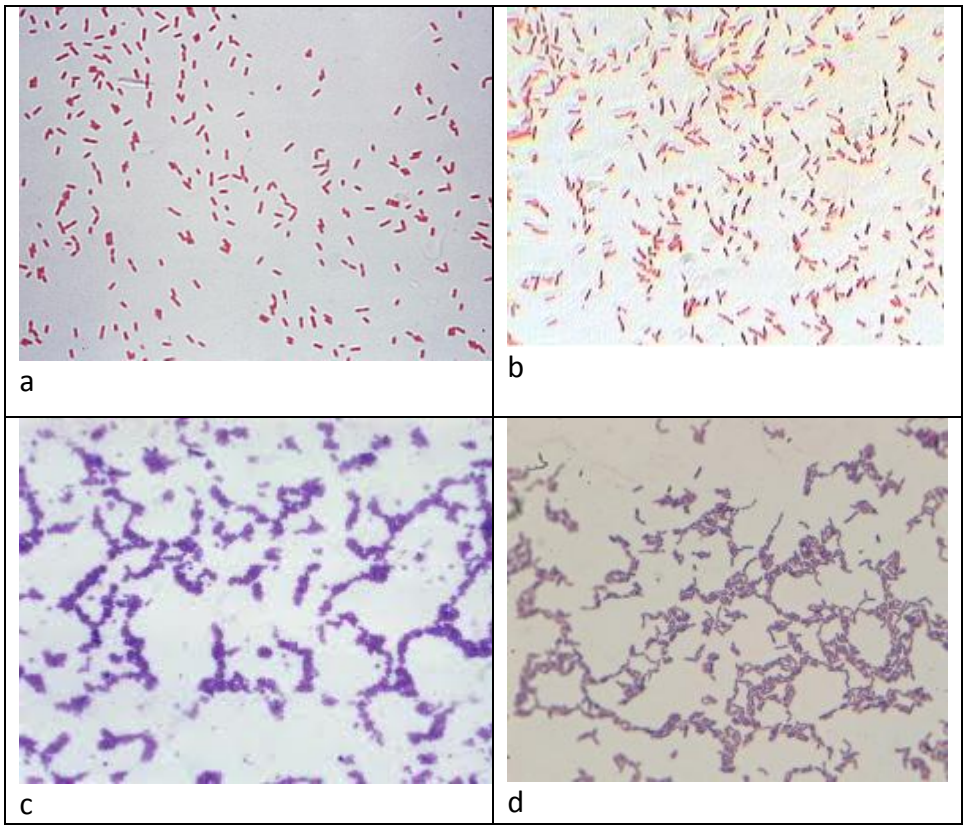


Fig-2: Showing Gram's straining reactions; a) *Pseudomonas;* b) *E.coli;* c) *Stahylococcus;* d) *Lactobacillus.*

Further, the isolates were processed for biochemical characterization based upon different standard methods and results obtained further confirmed the presence of *Escherichia coli* as indole, MR and catalase showed positive whereas VP and citrate were negative; *Pseudomonas as* MR, VP and catalase were positive whereas indole showed negative; *Staphylococcus* as MR and catalase gave positive result whereas indole and citrate were negative and VP may or may not be positive) (Figure 3; Table 4).

ISSN: 0975-8585

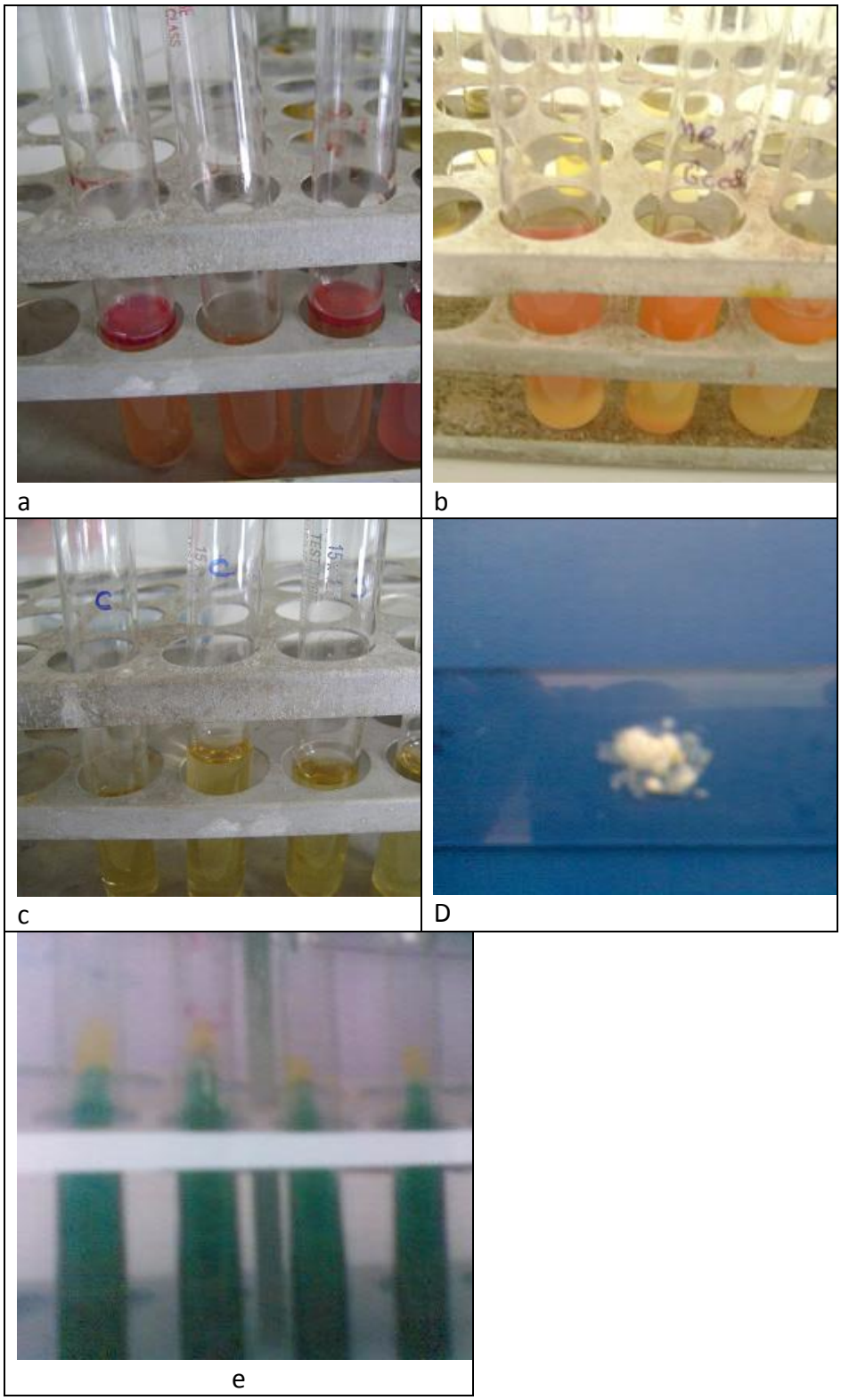


Fig-3: Showing biochemical tests of isolates (*E.coli, Pseudomonas, Staphylococcus, Lactobacillus)*; a) Indole tests- positive confirmed by presence of cheery red colour at the top; b) Methyl red tests – positive confirmed by presence of red colour; c) Voges proskaur test – negative confirmed by no formation of crimson red colour; d) Catalase tests- positive confirmed by formation of bubble; e) Simmon citrate test – negative confirmed by no change in colouration.

ISSN: 0975-8585

Table 4. Summary of Biochemical Characterization of microorganisms (*E.coli, Pseudomonas, Staphylococcus, Lactobacillus*) isolated from samples (Dairy products)

S. No	Indole test	MethylRed (MR)	Voges Proskaur (VP)	Citrate test	Catalase test
Observation	Red coloured ring on top of test tube	Red colouration throughout the tube	No crimson red colour	No change in colour	Production of bubbles
1.	+	+	_	_	+
2.	+	+	_	_	+
3.	+	+	_	_	+
4.	+	+	_	_	+
5.	+	+	_	_	+
6.	+	+	_	_	+
7.	+	+	_	_	+
8.	+	+	_	_	+
9.	_	_	_	_	_
10.	_	_	_	_	_

It is worth to mention that out of 10 samples taken for the study, 8 samples showed positive results. Two samples showed negative results as nothing was isolated from them. The samples which showed negative results were found to formed yellow ring near the surface of medium in case of indole test but we could not get further (Table 4).

The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food born disease, is not significant if *E. coli* is normally a ubiquitous organism [10], yet the pathogenic strains if present could be harmful to consumers. *S. aureus* on the other hand, releases a toxic chemical, enterotoxin. As little as 1.0 µg of the toxin in contaminated food produces symptoms of illness. Other bacterias also, in excess, in the diet may cause more or less harmful effects.

As milk is one of the most important nutrients and protein dense food, because it is an excellent source of nine essential nutrients and casein, a major milk protein. Further, dairy products like curd and paneer, khoya made from milk and their consumption plays a significant role in the supply of important nutrients and protein required for good health. These milk products are very essential, especially, in the Indian diet; therefore their contamination can cause varied health hazards.

WHO's (2007) was reported that, it is important to handle food in such a way that the microorganisms present do not have a chance to multiply and to prevent food from becoming contaminated with other microorganisms by:

1. Wash and dry hands before preparing any food and after handling foods (milk, curd, etc).
2. Ensure that food preparation areas and equipment are clean.

3. Protect kitchen areas or restaurants and food from insects, pests and other animals.
4. People with gastrointestinal illness, such as vomiting or diarrhoea, should not handle food intended for consumption by others.

The present study as well indicated that strict preventive measures should be adopted to ensure contamination free milk products for the good health of all consumers. For this, consciousness and care is required from the point of generation to the point of consumption of these widely consumed milk products.

REFERNCES

[1] Anjum MS, Lodhi K, Raza AA. In Pakistan Economy Analysis network project Islamabad 1989; 2: 1131- 1135.
[2] Thapa TB Food and Agriculture Organization of the United Nations;2000: Rome, Italy.
[3] Harper EB, Rittenhouse TAG , Semlitsch RD Conserv Biol 2008; 22:1205-1215.
[4] Boylston et al. International Dairy 2004 Jour; 14: 375-387.
[5] Ong L, Shah NP. Food Science and technol 2009; 42: 1260-1268.
[6] Ono K and Yamamoto K. Japan International Food Microb Jour 1999; 47: 211-219.
[7] Bichai F, Payment P, Barbeau B. Canadian Microb Jour 2008; 54**:** 509-524.
[8] Aneja KR. In New Age International (P). Ltd Publishers New Delhi Fourth edition. 2005.
[9] Bergey DH, Holt JG, Krieg NR, Sneath PHA; 1994: Williams and Wilkims, Baltimore, Unites States.
[10] Hahn G Int Dairy Federation; 996: 67–83.