# QUALITY ANALYSIS OF MILK WITH REFERENCE TO MICRORGANISM & PRODUCTION OF BACTERIOCINS.

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## *Abstract*

Milk contributes a greater number of essential nutrients for human nutrition than any other single food, some in relatively large amounts. It is an outstanding source of calcium, which is needed all through life for healthy bones and teeth. It also supplies many other vitamins including riboflavin and when fortified, vitamin–D which helps structural and tissue development. The protein in milk is one of the best quality proteins that any food offers. Due to this highly nutritious nature of milk, it serves as an excellent growth medium for a wide range of microorganisms


**Keywords**: Coliform test,Standard Plate count,Phosphatase test,MBRT test,Bacteriocins.

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## INTRODUCTION

Milk is an important food of diet of vast population on earth, due to its high nutritional value for human beings[2]. It is considered as a complete food and it contains proteins, fat, carbohydrates, minerals, vitamins and water and is also a excellent medium for the growth of microorganisms when suitable temperature exists [2].A variety of pathogenic organisms may gain access in to milk products from different sources and cause different types of food borne illnesses. Milk products may carry toxic metabolites of different organisms growing in it. Ingestion of such products, contaminated with these metabolites, cause infection or food poisoning. This happen because large volume of these products are produced in unorganized sector, unbranded, with little precautions of food safety and quality. Most of the indigenous milk products are prepared by traditional methods in the unorganized industrial sector. Such methods often bring about contamination of various microorganisms including pathogens. Milk drawn from a healthy animal contains several hundred to several thousand microorganisms[7]. Microbes can degrade casein, lactose and milk fat, if milk may get contaminated with various pathogenic microbes, which may actually be due to contaminated water, containers, handling and variable temperature conditions[2].

The introduction of a few pathogens into milk becomes a much more serious problem because of the ability of these substances to support tremendous increases in bacterial numbers[8]. Products of Microbial Growth are Enzymes, Decomposition products (fats, proteins, and sugars), Pigments and Toxins. Certain microorganisms produce chemical changes that are desirable in the production of dairy products such as milk. It is therefore, important to know the types of microorganisms present in milk, their control and use for beneficial purposes. Microorganisms that are generally present in milk are LAB and coliforms. The raw milk contains higher number of micro flora probably due to contamination from the animal. Bacteria found in manure, soil and water may enter milk due to dairy utensils and milk contact surfaces. Such contamination can be reduced by clipping the cow, and washing the udder with water or a germicidal solution before milking. Contamination of cow with manure, soil, and water may also be reduced by paving and draining Barnyards, keeping cows from stagnant pools, and cleaning manure from the barns or milking parlours. Bacteria multiplies at $0^0$C also with slow rate and responsible for spoilage of milk.The coliform bacteria are able to grow well in a variety

of substrates and to utilize a number of carbohydrates and some other organic compounds as food for energy and a number of fairly simple nitrogenous compounds as a source of nitrogen[8]. Coliforms are facultative anaerobes with an optimum growth at 37° C. Coliforms are indicator organisms, they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also can cause rapid spoilage of milk because they are able to ferment lactose with the production of acid and gas, and are able to degrade milk proteins. They are killed by HTST (pasteurization) treatment; therefore, their presence after treatment is indicative of contamination. *Escherichia coli* is an example belonging to this group.

Many milk-borne epidemics of human diseases such as tuberculosis, typhoid, diphtheria, dysentery have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies [8]. The bacterial count of milk is used to measure its sanitary quality and most grading of milk is on the basis of some method for estimating numbers of bacteria (Collins et al., 1995), such as the standard plate count (SPC) which determines the total number of bacteria in a sample that can grow and form countable colonies when incubated aerobically at $32^0$C for 48 hours [3]. Generally SPC values for raw milk according to Bramley and Mckinnon (1990) that counts greater than $10^5$cfu/ml are indicative of serious faults in production hygiene [2]. Microbial contamination occurs from within the udder because as raw milk leaves the udder of healthy cows it contains very low numbers of micro organisms (Kurwell, 1973) [3]. However, cows with mastitis have the potential to shed large numbers of micro organisms into the milk (Bramley and Mckinnon, 1990) [3].Thus, microbiological and biochemical examination of milk is essential to find the degree of contamination with the dictions and enumeration of indicator organisms [8]. In the present study the quality of 12 milk samples in terms of standard plate count, oxidation-reduction potential, presumptive coliform test, etc., was determined. Bacterial contamination of milk was checked by the MBRT test [9].The project work was experimental in nature with an emphasis on microbiological quality of milk.

Enumeration of various bacteria was done to find out their number by different methods eg. Standard plate count. Yeasts and moulds in milk samples can cause souring of milk samples and make them non suitable for drinking. Dye reduction methods depend upon the ability of microorganisms to alter the oxidation-reduction potential of the medium. They are

in consequence a measure of the activity of microorganisms in the test system rather than of the numbers in the sample. Suitable indicator dye includes methylene blue. The length of time taken to reduce the dye depends on the mass and activity of bacteria present in the sample.the greater the number present shorter the time required for reduction. **Phosphatase is an enzyme present in raw milk samples.** All raw cows' milk contains phosphatase which is inactivated by effective pasteurisation[15]. This is the basis of the legal phosphatase test, used to demonstrate that the pasteurisation has been effective[15].**This enzyme is destroyed by adequate pasteurization of milk which in terms of heat tolerance is similar to Rickettsia,cxiella burnetii(fever organism) or Mycobacterium bovis (tuberculosis bacterium).So, its presence in milk samples indicates the inadequate pasteurization and presence of other harmful bacterium.**

Lactic acid bacteria are able to ferment lactose to lactic acid. Some examples are: lactococci (*L. delbrueckii Lactococcus lactis),* lactobacilli (*Lactobacillus casei, L.delbrueckii L. delbrueckii), Leuconostoc.* Present study also includes isolation of lactic acid bacteria's which are responsible for producing bacteriocin toxin in milk. Bacteriocin is an antibacterial substance, such as colicin, produced by a strain of bacteria and harmful to another strain within the same family. These are the proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Their most important habitat is in untreated milk. They are typically considered to be narrow spectrum antibiotics.They are phenomenologically analogous to yeast and paramecium killing factors, and are structurally, functionally, and ecologically diverse. The bacteriocins produced by LAB offer several desirable properties that make them suitable for food preservation: (i) are generally recognized as safe substances, (ii) are not active and nontoxic on eukaryotic cells, (iii) become inactivated by digestive proteases, having little influence on the gut microbiota, (iv) are usually pH and heat-tolerant, (v) they have a relatively broad antimicrobial spectrum, against many food-borne pathogenic and spoilage bacteria, (vi) they show a bactericidal mode of action, usually acting on the bacterial cytoplasmic membrane: no cross resistance with antibiotics, and (vii) their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation.

Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria

following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body. Bacteriocin from food grade lactic acid bacteria(LAB) appear to qualify as ideal food biopreservative primarily because they have proven non toxic to humans, do not alter the nutritional properties, effective at low concenterations,active under low concenteration, active under refrigerated storage and can be used even in the preservation of foods by the use of bacteriocins produced by lactic acid bacteria. Bacteriocins have also been suggested as a cancer treatment. They have shown distinct promise as a diagnostic agent for some cancer, but their status as a form of therapy remains experimental and outside the main thread of cancer research.

The importance of microbiology to the dairy industry has been demonstrated by food borne illness associated with consumption of milk that had been contaminated with pathogenic organisms and toxins.Undesirable microorganisms constitutes the microbiological analysis of milk designed to evaluate quality and to ensure safety and regulatory compliance. So, the present study is thus been designed to assess the milk quality with special reference to lactic acid bacteria

## 3.0 MATERIALS and METHODS

**3.1 MATERIALS:** Glassware (Borosil), Cellulose Acetate Filter Paper, Dialysis Bag, Sabouraud Dextrose Agar( SDA), Mueller Hinton Agar (MHA), Eosine Methylene Blue (EMB) Agar, Nutrient Agar, M17 Agar, MRS Agar, Mackonkey broth, Sugars (Ribose, Galactose, Trehalose, Xylose, Arabinose ), Potassium Phosphate Buffer, Strains of Lactic Acid Bacteria *Lactococcous lactis* subspecies lactis MTCC440 & *Lactobacillus plantarum* MTCC1407 (IMTECH Chandigarh,India), Methylene Blue Dye, Hydrogen Peroxide .All the chemicals,reagents and bacteriological media used were of analytical grade from LOBA Chemie Pvt. Ltd.

### 3.2 METHODS

**3.2.1 Collection of Milk samples:** 15 raw milk samples from Local dairy of Kapurthala, Jalandhar & Phagwara region were collected.Various tests to check the quality of milk samples was then carried out.

**3.2.2 Standard plate count (SPC)**: This method was used to determine the total bacterial colony count in the samples. Dilutions of $10^{-8}$ were prepared and 1ml of each dilution was mixed with aerobic plate agar (nutrient agar) in petri dishes. After incubation at 37°C for 48 hours, the colonies were counted.

**3.2.3 Enumeration of *L.delbrueckii*:** This method was used to find out the number of *L.delbrueckii*. $10^{-8}$ sample dilutions was prepared and inoculated in M17 agar medium. Colonies were counted manually after incubation of 24-48 hrs at 37°C.

**3.2.4 Coliform count:** This test is used to find out whether the coliforms are present in milk samples or not. Presumptive, confirmed and completed coliform tests were performed. For presumptive test 15 tubes were taken (5 boiling test tubes and 10 normal test tubes).Durham tubes were added to each test tube.Then 10 ml mackonkey broth were taken double strength in boiling test tubes and single strength in normal test tubes. It was further followed by 10 ml sample in 5 boiling test tubes,1ml sample in 5 normal test tubes & 0.1ml sample in 5 normal test tubes.These tubes then were incubated for 24- 48 hours at 37°C and observed for the acid production and gas formation . Acid production was confirmed by color change i.e. from reddish brown to yellow and gas production was confirmed by bubble formation and appearance of head space in durham tubes. The positive samples were further streaked on Eosine Methylene Blue (EMB) agar and incubated for 24-48hrsat 37°C. This was done to confirm the bacteria present in milk samples.

**3.2.5 Yeast and Mould count**: This test was performed for yeast and mould count. Sabouraud Dextrose agar medium plates were prepared and 1 ml ($10^{-8}$ dilution) of each samples was inoculated on the plates by spread plate method. These plates were then incubated at 30°C for 2-5 days and after the incubation period the colonies were counted manually.

**3.2.6 Methylene blue reduction test:** 10 ml of each milk sample was added to sterilized glass tubes followed by addition of 1ml of methylene blue. Test tube containing boiled milk was taken as a control. Tubes were placed in water bath at 37°C in an inverted position for incubation.

Time taken for methylene blue discoloration was recorded. Each sample was checked for discoloration after 30 min.

**3.2.7 Phosphatase test:** 1ml of each milk sample was taken in sterilized test tubes and 1ml of boiled milk was taken as a control. 5ml of buffer (sodium carbonate bicarbonate buffer , pH 7) was added in both the test tubes containing raw milk and boiled milk and kept for incubation in water bath for 2 hrs at 40-50°C. The tubes were removed and colors in both the test tubes were compared.

**3.2.8 Isolation of Lactic Acid Bacteria:** All the samples were spread plated on MRS,M17,MRS+1% Calcium carbonate agar media at a dilution of $10^{-10}$ **.** All the sample plates were then incubated at 37°C .The plates were then observed for the presence of lactic acid bacteria.

**Test for characterization of lactic acid bacteria:** To confirm the isolates as lactic acid bacteria following tests were performed:

**Gram Staining:** Smear of colonies were heat fixed on the slide with the help of a Bunsen burner . Addition of primary stain which is crystal violet was done on the slide and allowed to stay for 1 minute. Slide was then rinsed with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet. It was followed by addition of Gram's iodine for 1 minute. Slide was rinsed again with alcohol for approximately 3 seconds and rinsed with a gentle stream of water. secondary stain, safranin was then added to the slide and allowed to stay for 1 minute. Washing of slide with a gentle stream of water was done for a maximum of 5 seconds.

**Catalase Test:** A sterile toothpick was used to smear a portion of the colony onto a microscopic slide. A drop of hydrogen peroxide was placed onto the smear. The microscopic slides were then observed for bubbles formation. Bubbles formed confirm that the colony is catalase positive.

**Sugar fermentation test:** 5ml of nutrient broth was taken in test tubes to which 0.5 mg of different sugars (Ribose,galactose, trehalose, xylose, and arabinose) were added. A pinch of bromophenol blue was added in each of the above test tube .A portion of the colonies that tested

positive for catalase test were incubated at 37°C for 24-48hrs and the tubes were checked for acid and gas production..

**3.2.10 BACTERIOCIN PRODUCTION:**

**3.2.10.1 PROCUREMENT OF REFERENCE LACTIC ACID BACTERIAL STRAINS:**

*Lactococcus lactis* subspecies lactis MTCC 440 and *Lactobacillus plantarum* MTCC 1407 procured from IMTECH Chandigarh was taken as a reference for comparison with antimicrobial activity of isolate.

**3.2.10.2 Checking the antimicrobial acitivity by Dialysis:** purification of bacteriocin was done as per the method followed by S.T.ogunbanwo et al [1]. Isolate and strains inoculated in MRS broth was taken and centrifuged at 10000 rpm for 20 min. at 4°C. pH of supernatant was set to 7 with the help of 1M sodium hydroxide to exclude the activity of organic acid. It was then followed by filtration of supernatant with the help of 0.2Mm pore size cellulose acetate filter. Dialysis bags were prepared for isolate & each strain. Purification of isolate with ammonium sulphate precipitation was done by preparing two concenteration i.e. 20% and 40% of isolate and strain which was then followed by addition of ammonium sulphate and centrifugation at 20000 rpm for 1hr. Precipitates were resuspended in 25ml of 0.05M potassium phosphate buffer. dialysis was again done. Then the antimicrobial activity of resulted isolates was cheked against two hosts *E.coli* and *Vibrio Parahaemolyticus*. Mueller Hinton Agar was prepared and poured in petriplates .After solidification of agar hosts were swabbed on it. 20% and 40% concenteration of isolate and strains were added in wells. After incubation of 24 hrs plates were observed to check the hallow zone formation.

**4.0 RESULTS & DISCUSSION:**

**4.1 Standard Plate Count:** Out of 15 milk samples the microbial colonies were found to me high in five milk samples (sample 6,7,8,12,13) with number of colonies more than 200 and the colony content was low in eight milk samples (sample 1,3,5,9,10,11,14,15) with colony number

less than 200 . The results showed that milk sample 8 was highly contaminated with 450 colonies & there was minimum contamination in sample 1 with 59 (Table 6.1, Photograph 7.1a, 7.1b).Out of 15 milk samples Sample 2 and 4 showed confluent growth which means colonies were uncountable.

**4.2 Enumeration of *L.delbrueckii*:** Number of lactic acid bacteria i.e. *L.delbrueckii* was found in high number in eight milk samples (sample 1,2,3,4,5,8,9,12) with number of colonies more than 200.Sample number 3 was found to have 607 number of colonies which was maximum out of rest of the milk samples whereas colonies number was minimum in sample 13 (Table 6.2, Photograph 7.2a, 7.2b).Sample 6 showed confluent growth.

**4.3 Coliform Count:**

**Presumptive test** : The results showed that the acid & gas production occurred in each sample which was confirmed by color change from reddish brown to yellow and formation of bubble in durham tubes (Photograph 7.3a). Results showed that the acid & gas production was maximum in sample 1, 3 & 12 (Table 6.3.1) with MPN index value of 500,280 and 280.rest of the milk samples were also found to show acid and gas production but in less number of tubes.

**Confirmed test** : This test was performed for each sample by inoculating them on to the EMB agar, where three morphologically distinct colonies were obtained (Table 6.3.2, Photograph 7.3b.7.3c). On microscopic examination of the colonies, it was found that the pinkish mucoid colonies were of rod shaped based upon colony characteristics and microscopic examination , the above mentioned colonies might be *Enterobacter* sp. The second type of colonies was spherical, with concave elevation. *E.coli* was detected by presence of green metallic shine on EMB Agar in milk sample 6 only. This indicates poor quality of sample 6 as compared to other milk samples.

**4.4 Yeast & Mould Count:** Yeast & Moulds were found to be maximum in sample 9 with 262 number of colonies and minimum in sample 2 with 44 colonies number (Table 6.4, Photograph7.4a, 7.4b) which indicates that sample 9 was highly contaminated with yeasts and moulds. Confluent growth was observed in sample 3, 5, & 6 (Photograph 7.4c, 7.4d, and 7.4e).Sample 12 was also found to be contaminated with 232 (more) number of colonies.Rest of

the other milk samples were having colonies less than 200 which shows there appropriate quality.

**4.5 Methylene blue reductase test:** The results of this test was based on reduction of methylene blue dye by the bacteria present in milk samples. More is the number of bacteria present in samples lesser will be the time taken to reduce methylene blue. The results revealed that sample 2, and 4 were excellent to sustain the time period of 400 minutes (Table 6.5) so, can be graded as good milk samples. Whereas sample 6 and 8 were not able to sustain more time period so, they are graded as poor milk samples. Raw milk samples were found to be discolored in less time as compared to boiled milk (Photograph 7.5).

**4.6 Phosphatase test:** Results showed that phosphatase enzyme was present in all the 12 milk samples. This confirms that the milk samples were not pasteurized. The presence of this enzyme in all the milk samples indicates that these samples may also contain *Cxiella burnetii* (fever organism) and *Mycobacterium bovis* (tuberculosis bacterium). After the incubation of 2 hrs at 40-50°C it was found that raw milk sample showed a dark color as compared to boiled milk sample (Photograph 7.6).

**4.7 Isolation of lactic acid bacteria:** Lactic acid bacteria was isolated when the milk samples were inoculated on MRS containing 1% calcium carbonate medium. One colony in milk sample 9 was found to be gram positive cocci under microscope & morphologically it was Creamish moist (Table 6.6). On further performing tests the bacterial colony was characterised as colony of lactic acid bacteria.

**4.8 Identification of Lactic acid bacteria:**

**Gram staining:** Colonies taken were gram stained to support the characterization of lactobacilli and lactococci. Under microscope out of 53 colonies 52 were appeared violet in colour indicating gram positive LAB (Table 3, photograph ii).

**Catalase test:** No bubbling was shown by this colony when it is treated with hydrogen peroxide which confirms that the colony was catalase negative (Photograph 7.7).

**Sugar fermentation test :** Results of this test showed that bacteria produced acid when reacted with different sugars. This bacterium was then inoculated in MRS broth for revival.Two strains of Lactic acid bacteria *Lactococcous lactis* subspecies lactis MTCC440 & *Lactobacillus plantarum* MTCC1407 was taken as a control against this isolate (Photograph7.8).

**4.8 ANTIMICROBIAL ACTIVITY:** The antimicrobial activity of two isolates and two standard strains was studied. The strains & isolate were subjected to dialysis in the potassium phosphate buffer (Photograph 7.9a). Their degree of inhibition against test pathogens *E.coli* and *V. parahaemolyticus* was observed . The culture supernatants of both isolates and strains yielded zone of inhibition against test pathogens. The inhibition zone diameters ranged from 0.1cm to 2cm (Table 4). The highest diameter (2 cm) was recorded for culture supernatants of strain 440 on *V. parahaemolyticus*. *Vibrio parahaemolyticus* & *E.coli* were taken as a host. Results showed that the diameter of inhibition zone of strain 440 was 1cm & 1.1cm against *Vibrio Parahaemolyticus* & *E.coli*. Strain 1407 was found to produce inhibition zone of diameter 0.9cm & 1.5cm against *Vibrio Parahaemolyticus* & *E.coli*. Moreover isolate of lactic acid bacteria was found to show inhibition zone of diameter 1.2cm & 1.5 cm against both the host (Table 6.7).After first dialysis pellet & supernatant both were cheked for production of antimicrobial activity. Pellet of isolate showed inhibition zone of diameter 0.4cm and 0.8 cm against *Parahaemolyticus* and *E.coli* host. Filtrate of isolate showed zone of 0.7cm against both the hosts. Both the strains and isolate showed inhibition zone of varying diameter (Table 6.8).After the second dialysis two concenteration i.e 20% and 40% of supernatant and pellet was also checked(Table 6.9, Photograph 7.9b, 7.9c).These results assures the antimicrobial activity of strains & isolate.

**5.0 Conclusion**

The present study aimed at microbiological examination of raw milk samples and studying the antimicrobial activity of lactic acid bacteria.microbiological examination of a total of 15 raw milk samples were analysed. Ultimately from this analysis of milk samples of different reputed local dairies of Jalandhar, Kapurthala and Phagwara region we came to know that the overall quality of the milk was up to the mark. The results of standard plate count showed that milk sample 8 was highly contaminated and there was minimum contamination in sample 1. Number of *L.delbrueckii* was found to be maximum in sample 3 and minimum in sample 13. Coliform count showed that the acid and gas production was maximum in sample 1,3,11. *Pseudomonas* and *Enterobacter* were found in most of the milk samples whereas *E.coli* was found in sample 6 which shows that the sanitary quality of those samples was not good. Yeast and Moulds were found to be more in sample 9 & lowest in sample 2. MBRT test revealed that sample 2, 4 and were excellent to sustain the time period of 400 minutes and graded as GOOD samples. Sample

6 and 8 showed discoloration in very less time, which indicates their poor quality. Raw milk was found to show discoloration in less time as compared to boiled milk. Phosphatase enzyme was found to be present in all the milk samples which indicate the presence of other fever or tuberculosis causing bacteria. Lactic acid bacteria was isolated when the milk samples was inoculated on medium containing MRS + 1% calcium carbonate. Catalase and sugar fermentation test of colony isolated confirms that the colony was of lactic acid bacteria. Two strains of Lactic acid bacteria *Lactococcous lactis* subspecies lactis MTCC440 & *Lactobacillus plantarum* MTCC1407 was taken as control with respect to the isolate were taken as a control with respect to the isolate. The isolate of lactic acid bacteria showed inhibition zone against host organisms *Vibrio Parahaemolyticus* & *E.coli* after the process of dialysis which confirmed the strains and isolate showed antimicrobial activity against hosts.

## 6.0 TABLES:

### 6.1 STANDARD PLATE COUNT:

| MILK SAMPLES | NUMBER OF COLONIES |
|---|---|
| 1 | 59 |
| 2 | CONFLUENT |
| 3 | 199 |
| 4 | CONFLUENT |
| 5 | 62 |
| 6 | 251 |
| 7 | 253 |
| 8 | 450 |
| 9 | 115 |
| 10 | 103 |
| 11 | 133 |
| 12 | 263 |
| 13 | 221 |
| 14 | 112 |
| 15 | 134 |

**6.2 ENUMERATION OF *L.delbrueckii*:**

| MILK SAMPLES | NUMBER OF COLONIES |
|---|---|
| 1 | 502 |
| 2 | 371 |
| 3 | 607 |
| 4 | 401 |
| 5 | 451 |
| 6 | CONFLUENT |
| 7 | 144 |
| 8 | 331 |
| 9 | 224 |
| 10 | 97 |
| 11 | 121 |
| 12 | 201 |
| 13 | 55 |
| 14 | 233 |
| 15 | 132 |

| SAMPLES | NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF 5 OF<br>10ML EACH 1ML EACH 0.1ML EACH | MPN INDEX PER 100ML |
|---|---|---|
| 1 | 5 5 2 | 500 |
| 2 | 5 2 2 | 90 |
| 3 | 5 4 3 | 280 |
| 4 | 4 3 1 | 33 |
| 5 | 4 3 1 | 33 |
| 6 | 5 3 2 | 140 |
| 7 | 5 3 1 | 110 |
| 8 | 5 4 2 | 220 |
| 9 | 5 2 2 | 90 |
| 10 | 5 4 1 | 170 |
| 11 | 5 3 3 | 170 |
| 12 | 5 4 3 | 280 |
| 13 | 4 3 1 | 33 |
| 14 | 4 3 1 | 33 |
| 15 | 5 2 2 | 90 |

| MILK SAMPLES | NUMBEROF COLONIES |
|---|---|
| 1 | 61 |
| 2 | 44 |
| 3 | CONFLUENT |
| 4 | 59 |
| 5 | CONFLUENT |
| 6 | CONFLUENT |
| 7 | 47 |
| 8 | 187 |
| 9 | 262 |
| 10 | 62 |
| 11 | 152 |
| 12 | 232 |
| 13 | 56 |
| 14 | 67 |
| 15 | 89 |

**6.4 YEAST & MOULD COUNT**:

**7.0 PHOTOGRAPHS:**

**7.1 STANDARD PLATE COUNT:**

**a)**


**b)**

## 7.2 ENUMERATION OF *L.delbrueckii*


**a)**


**b)**

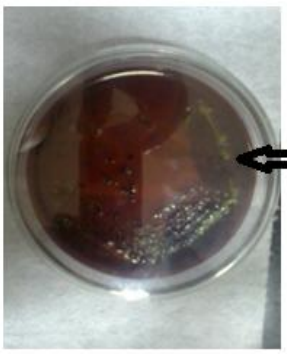
## 7.3 COLIFORM TEST:

**a)**

Yellow color indicates the production of acid

**b)**

pinkish mucoid colonies & purple colonies of Enterobacter & Pseudomonas



E.coli gives metallic green color on Eosine Methylene Blue agar

**7.4 YEAST & MOULD COUNT:**


**a)**

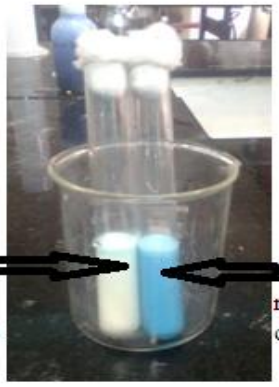

**b)**


**c)**


**d)**


**e)**

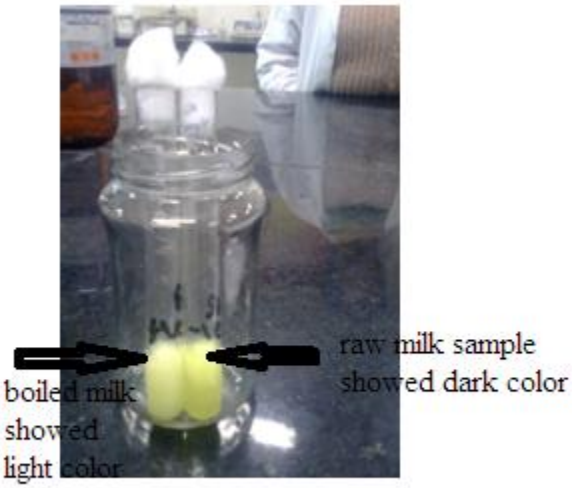
**7.5 MBRT TEST:**



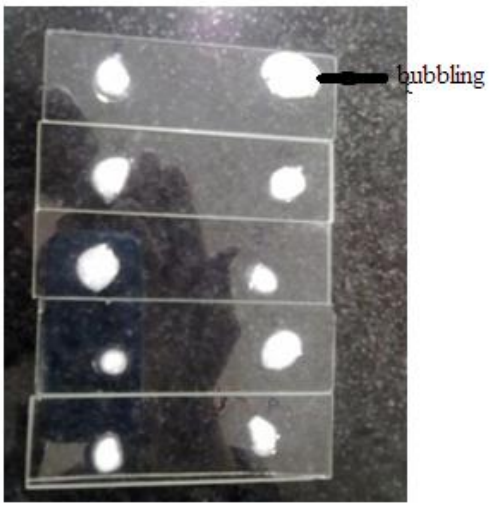
raw milk reduce methylene blue & show change in color in less time

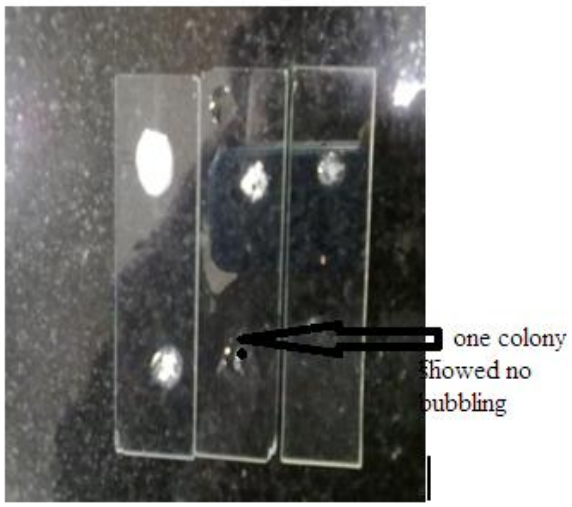
boiled milk shows no or very less change in color

**7.6 PHOSPHATASE TEST:**



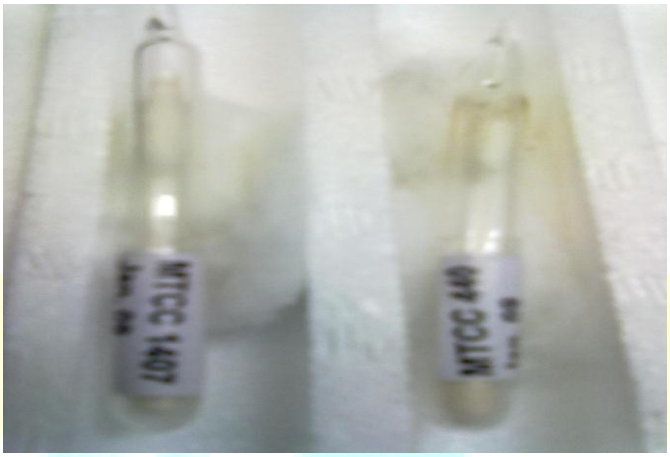

**7.7 CATALASE TEST:**



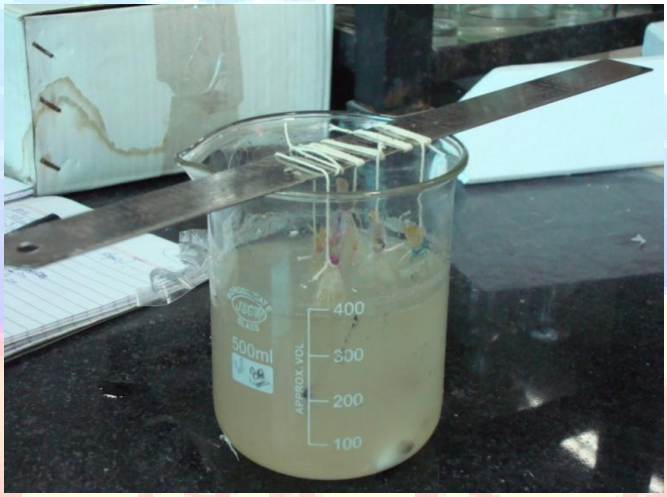

Lactic acid bacteria show no bubbling when treated with hydrogen peroxide. Only one colony showed no bubbling.

## 7.8 STRAINS OF LACTIC ACID BACTERIA

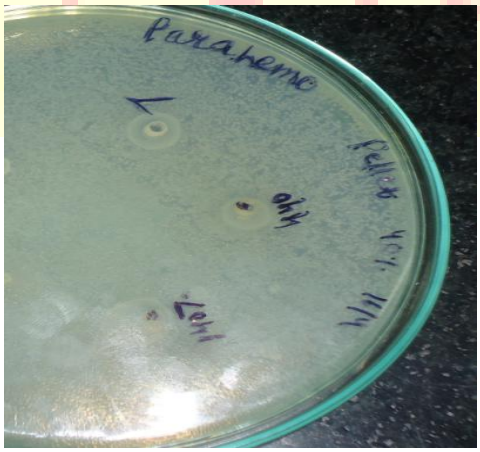


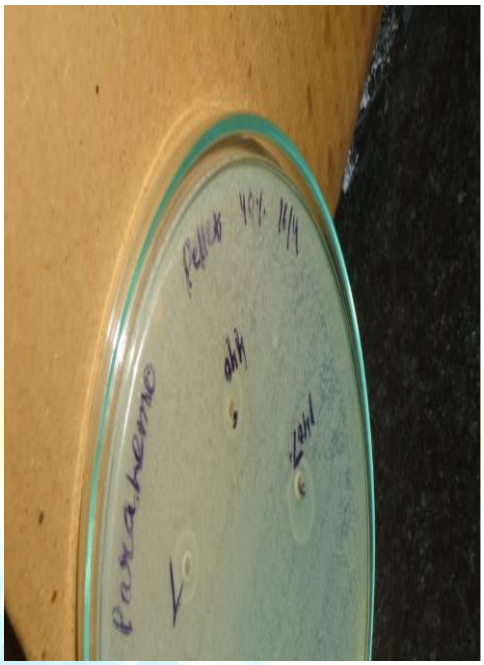

## 7.9 ANTIMICROBIAL ACTIVITY:

a)




b)



c) 

## Conclusion

Ultimately from this analysis of milk samples of different reputed local dairies of Jalandhar and kapurthala region we come to know that the overall quality of the milk is up to the mark. The SPC of the local samples was exceeding the standard quality limits and the *Pseudomonas* and Enterobacter were found in the branded samples which shows that the sanitary quality of those samples was not good. Coliforms were found in the Local dairy samples . MBRT test for all the branded sample shows that the pasteurized milk stays up to 400 minutes which shows its excellent quality where as the local dairy milk starts reducing Methylene blue after 300 minutes, which shows its fair quality.

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