

Original Research Paper

Zoology

ISOLATION OF SELECTED MICROORGANISMS FROM BUFFALO RAW MILK

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ABSTRACT Milk is our primary source of calcium, phosphorus, riboflavin and vitamin D, and is among the top three sources of protein, vitamins A and B12, potassium, and zinc. Buffalo milk is mostly consumed by people due to its consistency and availability. In the present studied we collected samples of buffalo milk and isolated microbial stains. In this study we had identified bacterial species representing the genus lactobacillus, Staphylococcus aureus and Escherichia coli were isolated from buffalo raw milk sample.

KEYWORDS : Buffalo milk, microorganisms, biochemical test

INTRODUCTION

The lacteal secretion obtained from one or more healthy milk producing animals example cow, goat, buffalo, and sheep including but not limited to the following low fat milk, skim milk, cream half and half dairy milk nonfat dairy milk, dairy cream, condensed or concentrated milk products cultured or acidified milk or milk products An opaque white or bluish – white liquid secreted by the memory glands of female mammals serving for the nourishment of their young. Buffalo milk may contain almost all the beneficial compounds found in other milks, e.g., proteins, peptides, fatty acids, vitamins, and other bioactive compounds. The liquid as secreted by cow, goat, buffalo or certain other animals and used by humans for food are as a source of butter, cheese, yougurt etc. Buffalo milk may contain almost all the beneficial compounds found in other milks, e.g., proteins, peptides, fatty acids, vitamins, and other bioactive compounds.

Fresh or raw milk is enrichment medium to support growth of contaminating microbes. Milk from healthy odder contains few bacteria but it picks up many bacteria from the time it leaves teat of buffalo until. This micro organisms are indicated of the both the manner of handling of milk from milking till consumption and isolation of milk. Milk produce under hygienic condition from healthy animals should not contain bacterial stain. In the present study microorganisms were isolated from buffalo raw milk

METHODOLOGY SAMPLE COLLECTION

In the present study domestic buffalo raw milk from different in Tirupati, chittoor district are collected in sterile bottles. Which are previously rinsed with distilled water and sterile it 70% alcohol. At the collection point the continuous were rinsed with the sample water before being used to collect sample.

The collected samples were placed in the refrigerators physical, chemical analyses were done by using the standard procedures. The microbial isolation was done by spread plate method on nutrient agar and a selective media for the identification. The final identification of resulted isolates was done by the biochemical test in accordance.

Serial dilution method:

The inoculums is subjected to serial dilution in a sterile liquid medium and a larger number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilutions. If we take cut 1 ml of the medium and mix with 9 ml of fresh sterile liquid medium we would then have hundred microorganisms is 10 ml. If we add 1 ml of this suspension to another 9 ml of fresh sterile liquid each ml would now contain a single micro organism in the medium and represents the pure culture of the microorganisms.

Spread plate method:

In this method the mixed culture of microorganism is not diluted in the melted agar medium, it is rather diluted in a series of tubes

containing sterile liquid, usually water are physiological saline. A drop of so diluted liquid from each tube is placed on the center of an agar plates and spread evenly over the surface by means of a sterilized bent glass rod.

The medium is now incubated in the colonies developed on the agar medium plates; it is found that there are some plates in which well isolated colonies grow. This happens as a result of separation of individual microorganisms by spreading over the drop of diluted liquid on the medium of the plate. The isolated colonies are picked up and transferred onto fresh medium to ensure purity. In contrast to pour plate method only surface colony develop in the method and the microorganisms are not required to with stand the temperature of the melted agar medium.

Sub culturing:

Sub culturing is the aseptic transfer of micro-organisms from a culture to fresh medium. The freshly inoculated medium is then incubated at the temperature appropriate for growing the organism.

Gram staining:

Gram staining is a method commonly used to determine the chemical makeup of the cell wall of bacteria. The cell wall can stain either positive or negative, depending on its chemistry.

Biochemical test:

IMVIC test:

Cultures have to grow for 24 to 48 hours at 37°C and the respective tests can be performed: tryptone broth (indole test), methyl red – Voges Proskauer broth (MR-VP broth), and citrate.

RESULTS AND DISCUSSION

The results studied were the classification the biochemical stain obtained in pure cultures from raw milk sample. The bacterial stain was retained mainly to the generic nomenclature known as stephylococcus aries.

Table: Results for the bacterial isolates in raw milk of buffalo

| Bacterial stain | Name of test | ISolate |
|--------------------------|-------------------|------------------------------------|
| Staphylo coccus aries | Gram stain | Gram negative cocci in clusters |
| Lacto bacillus | Gram stain | G+ve |
| E.coli | | |
| Biochemical test (IMVIC) | Reaction | Isolate |
| Indole test | Methyl red | Negative |
| Methyle red test | Colourless | Positive |
| vogus-poskaur test | Colourless | Positive |
| Citrate utilization | Orangish - yellow | Positive |

Domestic buffalo has an important place in agricultural production of Asia. Domestic buffalo in many regions in which they are raised

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are triple purpose milk animals as raw milk is important for making dairy products in households. A production of milk is particularly important, which in certain nations is used as a substitute for cooking. Every year is 82 millions litters of buffalo milk produced in the world, which is around 13 % of all the global milk. The biggest producer is India with 91 % of the buffalo milk. Milk composition differs between buffalo and cow. Buffalo milk contains a high concentration of all components, mainly protein and fat but there also difference in lactose, minerals and vitamins levels. The higher nutritional value brings good physical and chemical properties for use in the dairy industry. Milk borne pathogens can cause illnesses, and several other important microorganisms are also present in milk. The present study reveals the bacterial stains isolated from the milk sample by following the isolation techniques. The bacterial isolation were carried out on the basis of their microscopic characteristics observed through the cultural characters of given sample. In this study we had identified bacterial species representing the genus lactobacillus, Staphylococcus aureus and Escherichia coli isolated from buffalo milk sample. In these stains identified e.coli may cause diarrhea. Hygienic milk production practices, proper handling and storage of milk, and mandatory pasteurization has decreased the threat of milk borne diseases such as tuberculosis, brucellosis, and typhoid fever. There has been a number of food borne illnesses resulting from the ingestion of raw milk, or dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination.