Semester – III

17MBU312 FOOD AND DAIRY MICROBIOLOGY – PRACTICAL (4H – 2C)

Instruction Hours / week: L: 0 T: 0 P: 4 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 9 Hours

SCOPE

This paper provides necessary information on the food, dairy Microbiology in safety and quality perspective. Its importance in the prevention of contamination that might be caused by the microorganisms.

OBJECTIVES

- To encode the importance of the role of microorganisms in food industries both in beneficial and harmful ways.
- To obtain a good understanding of food and dairy products and become qualified as microbiologist in food and dairy industries.

EXPERIMENTS

1. MBRT of milk samples
2. Standard plate count of milk sample.
3. Alkaline phosphatase test to check the efficiency of pasteurization of milk.
4. Isolation of food borne bacteria from food products.
5. Isolation of spoilage microorganisms from spoiled vegetables/fruits.
6. Isolation of spoilage microorganisms from bread.
7. Preparation of yogurt.

SUGGESTED READINGS

1. Jay JM, Loessner MJ and Golden DA. (2005). Modern Food Microbiology. 7th edition, CBS Publishers and Distributors, Delhi, India.
2. Frazier WC and Westhoff DC. (1992). Food Microbiology. 3rd edition. Tata McGraw-Hill Publishing Company Ltd, New Delhi, India.
3. Adams MR and Moss MO. (1995). Food Microbiology. 4th edition, New Age International (P) Limited Publishers, New Delhi, India.
4. Gould GW. (1995). New Methods of Food Preservation. Blackie Academic and Professional, London.
5. Banwart JM. (1987). Basic Food Microbiology. 1st edition. CBS Publishers and Distributors, Delhi, India.
6. Davidson PM and Brannen AL. (1993). Antimicrobials in Foods. Marcel Dekker, New York.
7. Dillion VM and Board RG. (1996). Natural Antimicrobial Systems and Food Preservation. CAB International, Wallingford, Oxon.
8. Lund BM, Baird Parker AC, and Gould GW. (2000). The Microbiological Safety and Quality of Foods. Vol. 1-2, ASPEN Publication, Gaithersberg, MD.
9. Tortora GJ, Funke BR, and Case CL. (2008). Microbiology: An Introduction. 9th edition. Pearson Education.

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FOOD AND DAIRY MICROBIOLOGY - PRACTICAL (16MBU312)

Experiment No. 1

MBRT of milk sample

Aim

To determine the quality of the given milk sample based on the difference in the microbial load milk sample provided.

Principle

Methylene blue reduction test depends on the fact that the color imparted to the milk by adding a dye such as methylene blue will disappear more or less quickly that depends on the quality of the milk sample to be examined. Methylene blue is a redox indicator that loses its color under the absence of oxygen and is thought to be reduced. The reduction of oxygen in the milk is due to the production of reducing substance in the milk due to the production of reducing substance in the milk due to the enhanced rate of bacterial metabolism. The dye reduction time refers to the microbial load in the milk and the total metabolism reduction of the microorganism.

Materials and methods

Milk sample to be analyzed, screw cap tubes, test tube racks, pipettes (10ml and 1ml) and water bath (37 °C).

Preparation of methylene blue

The solution is prepared by boiling water in a brown stoppered flask the adding 1 methylene blue tablet to the flask of hot water and dissolve completely under hot condition. The solution may be stored in the stoppered brown flask to protect from sunlight.

Procedure

Transfer 10ml of each milk sample to approximately labeled test tube.

Add 1ml of redox indicator methylene blue to each test tube containing the milk samples.

Tighten the test tube mouth with stopper gently invert at about 4 or 5 times to ensure proper mixing of the methylene blue solution.

Heat the tubes in the water bath at 37 °C. Note the incubation time.

Prepared by Dr. P. Akilandeswari, Assistant Professor, Dept of Microbiology , KAHE

Experiment No. 2

Standard plate count of milk sample

Aim

To estimate the bacterial population in the given milk samples

Principle

The test employs a serial dilution technique for easy quantification of the microorganisms. The appropriate dilutions of the milk sample are mixed with a sterile nutrient medium that can support the growth of the microorganisms when incubated at a suitable temperature. Each bacterial colony that develops on the plate is presumed to have grown from one bacterium or clump of bacteria in the inoculum. The total number of colonies counted on the plate represents the number of viable microorganisms present in the sample tested.

Materials required

Test tubes, conical flask, nutrient agar, pipette 1 ml, milk sample.

Procedure

Prepare 99ml of sterile blank and 9ml water blank. Add 1ml of the given milk sample to 99ml of sterile water and consider it as stock solution. Take 1ml of stock solution using sterile pipette and serially diluted up to 10^{-7}. The samples were plated from 10^{-5}, 10^{-6} and 10^{-7} diluents on nutrient agar medium by spread plate method. Plates were incubated at 37 °C for 24hrs.

Prepared by Dr. P. Akilandeswari, Assistant Professor, Dept of Microbiology , KAHE

Experiment No. 3

Alkaline phosphatase test to check the efficiency of pasteurization of milk

Aim

This method is used to detect the contamination of pasteurized milk by unpasteurized (raw) milk, as well as, pasteurization confirmation.

Principle

Alkaline phosphatase is an enzyme naturally produced by mammary cells and present in cow's milk. The concentration of alkaline phosphatase can vary based on feed, season, breed of cows, stage of lactation, and milk yield. Alkaline phosphatase is inactivated at a higher temperature than is required to kill non-spore-forming bacterial pathogens. Decreased activity of alkaline phosphatase indicates that the milk has been exposed to enough heat to kill non-spore-forming bacterial pathogens. Alkaline phosphatase activity assays can therefore be used to determine if the milk was pasteurized properly and if the pasteurized milk has been contaminated with raw milk. Alkaline phosphatase is detected by its ability to cleave phenolphthalein monophosphate releasing phenolphthalein. Phenolphthalein is pink in alkaline conditions.

MATERIALS REQUIRED

Pipettes, test tubes, water bath, and incubators, etc.,

Test Reagent (Z132A)	
2-Amino-2-Methyl-1-Propanol	73.2gm
Phenolphthalein Monophosphate	3.9gm
Hydrochloric Acid	21.9ml
Final pH 10.0 +/- 0.3 at 25 ºC	

Standard Reagent (Z132B)	
2-Amino-2-Methyl-1-Propanol	73.5gm
Tartrazine	40.0mg
Phenolphthalein	10.0mg
Hydrochloric Acid	21.9ml
Final pH 10.0 +/- 0.3 at 25 ºC	

Color Developer (Z132C)	
Sodium Hydroxide	10.0gm
Deionized Water	100.0ml

PROCEDURE

A positive control and a negative control should be run with each test. To prepare the positive control add 0.2ml of fresh raw milk to 100ml of milk that has been heated at 95 ºC for 1 minute. To prepare the negative control heat 5ml of milk or 5gm of milk product to 95 ºC for l minute. For testing other dairy products, such as sour cream, ice cream, cheese, butter, etc., see listed references.

- Pipette 1ml of sample into each of the two test tubes. Label one tube "Test" and the other tube "Standard".
- Place the tubes in a water bath or incubator and warm to 37ºC.
- Add 1 drop (0.04ml) of Test Reagent to the tube marked "Test", and 1 drop (0.04ml) of Standard Reagent to the tube marked "Standard". Mix thoroughly by tapping the bottom of the tube.
- Incubate the tubes at 37ºC for 30 minutes.
- Add 1 drop (0.04ml) of Color Developer to each tube and mix thoroughly.
- Visually compare the color of the "Test" to the color of the "Standard".

INTERPRETATION OF RESULTS

A positive result is determined by a color in the "Test" tube that is pink or pinker than the color of the "Standard" control tube. This indicates that there is still alkaline phosphatase present in the milk and it has either not been pasteurized or has been contaminated by unpasteurized milk.

If the "Test" solution is white or less pink than the "Standard" controls it is interpreted as a negative test for the presence of alkaline phosphatase. This indicates that the alkaline phosphatase has been inactivated by pasteurization.

The "Standard" approximates pasteurized milk that contains 0.1% raw milk. The positive control consists of pasteurized milk that contains 0.2% of raw milk.

Experiment No. 4

Isolation of food borne bacteria from food products

Aim:

To isolate and analyze the type of food borne bacteria from the given sample of food products.

Principle:

Foods are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microorganisms. Contaminated foods are often inedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers. Some of the important food poison produced by bacteria is botulism (caused by Clostridium botulinum), Staphylococcal poisoning (Staphylococcus aureus) and enteric (E. coli) and (Salmonella typhimurium). This food poisoning is serious and sometimes fatal.

Materials required:

Sample: Food products

Medium: Nutrient agar medium

Sterile petriplates, inoculation loop, sterile pipettes, mortar and pestles.

Procedure:

- About 1 gm of the food sample to be tested was taken and placed in a clean mortar.
- The sample was then ground to a fine pulp with the help of a pestle.
- The sample was added into 99 ml of the sterile distilled water and considered as stock solution. Then 1 ml of the stock solution is added to 9 ml of sterile distilled water to obtain 10^{-1} dilution, the sample was serially diluted up to 10^{-7} dilution.
- Then spread plate technique was performed using nutrient agar plates for the bacterial isolation. 01 ml of the sample was used for spread plate technique.
- After 24 hours of incubation of the plates at 37 °C. The colonies were counted and the samples of food products can be used to judge the microbial quantity of the food samples.

Experiment No. 5

Isolation of spoilage microorganisms from spoiled vegetables and fruits

Aim:

To isolate and distinguish the spoilage microorganisms from spoiled vegetables and fruits.

Principle:

Foods are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microorganisms. Contaminated foods are often inedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers. Some of the important food poison produced by bacteria is botulism (caused by Clostridium botulinum), Staphylococcal poisoning (Staphylococcus aureus) and enteric (E. coli) and (Salmonella typhimurium). This food poisoning is serious and sometimes fatal. Microbial spoilage of vegetables and fruits may be due to plant pathogens, saprophytes. Organisms may be secondary invader and may enter a healthy vegetable or fruit in case of various rots and grow on its surface.

Materials required:

Sample: Spoiled vegetables and fruits.

Medium: Nutrient agar medium for bacterial isolation and Potato dextrose agar for fungal isolation, antibiotic (chloramphenicol).

Sterile petriplates, inoculation loop, sterile pipettes, mortar and pestles.

Procedure:

- About 1 gm of the spoiled vegetable (tomato, brinjal, carrot) and fruits (mango, pomegranate) to be tested was taken and placed in a clean mortar.
- The sample was then ground to a fine pulp with the help of a pestle.
- The sample was added into 99 ml of the sterile distilled water and considered as stock solution. Then 1 ml of the stock solution is added to 9 ml of sterile distilled water to obtain 10^{-1} dilution, the sample was serially diluted up to 10^{-7} dilution.
- Then spread plate technique was performed using nutrient agar plates for the bacterial isolation and potato dextrose agar for fungal isolation. 01 ml of the sample was used for spread plate technique.
- After 24 hours of incubation of the plates at 37 °C in the case of bacteria and 3-5 days of incubation at room temperature for fungi. The colonies were counted and the samples of vegetables and fruits can be used to judge the microbial quantity of the vegetables and fruits.

Experiment No. 6

Isolation of spoilage microorganisms from bread

Aim:

To isolate and distinguish the spoilage of microorganisms from bread.

Principle:

Foods are more susceptible to microbial decomposition because they are rich in nutrients that support the growth of microorganisms. Contaminated foods are often inedible and may also be the source of human diseases if contaminating organism are pathogens or toxin producers. Some of the important food poison produced by bacteria is botulism (caused by Clostridium botulinum), Staphylococcal poisoning (Staphylococcus aureus) and enteric (E. coli) and (Salmonella typhimurium). This food poisoning is serious and sometimes fatal. Molds are most common cause for spoilage of bread. The temperature attained in the baking procedure is usually high enough to kill the air during cooling from handling or from wrapper and usually initiate growth in the crease of the loaf and between the slice of the bread. Chief molds involved in the spoilage of bread are the bread molds, Rhizopus stolonifer, other molds are Pencillium expansum or Pencillium stolonifer, Aspergillus niger etc. Ropiness of bread is common in homr baked breads, especially during hot weather. It may be caused by bacterial species namely Bacillus subtilus or Bacillus licheniforms.

Materials required:

Sample: Bread

Medium: Nutrient agar medium for bacterial isolation and Potato dextrose agar for fungal isolation, antibiotic (chloramphenicol).

Sterile petriplates, inoculation loop, sterile pipettes, mortar and pestles.

Procedure:

- About 1 gm of the spoiled bread to be tested was taken and placed in a clean mortar.
- The sample was then ground to a fine pulp with the help of a pestle.
- The sample was added into 99 ml of the sterile distilled water and considered as stock solution. Then 1 ml of the stock solution is added to 9 ml of sterile distilled water to obtain 10^{-1} dilution, the sample was serially diluted up to 10^{-7} dilution.
- Then spread plate technique was performed using nutrient agar plates for the bacterial isolation and potato dextrose agar for fungal isolation. 01 ml of the sample was used for spread plate technique.

- After 24 hours of incubation of the plates at 37 °C in the case of bacteria and 3-5 days of incubation at room temperature for fungi. The colonies were counted and the samples of bread can be used to judge the microbial quantity of the bread.

Experiment No. 7

Yogurt production

Aim

To prepare yogurt from milk using *Lactobacillus* and *Streptococcus*

Introduction

Yogurt is produced by the fermentation of warm milk by *Lactobacillus bulgaris* and *Streptococcus thermophillus*. These two bacteria are able to grow at 40-45°C during which they produce lactic acid and various other by products that give its unique flavor.

Materials required

Cow milk, concentrated skim milk, non fat dry milk, whey lactose, sweatners – glucose and sucrose, high intensity sweetners eg) aspartains.

Stabilizers – gelatin, carbonyl methyl cellulose, lowest sugar, alginates, whey protein concentrate, starts culture, *Strptococcus salvarius* subsp thermophilus and *Lactobacillus delburki*

Procedure

100ml of the whole milk was taken in 500ml sterile beaker and boiled.

4gm of powdered milk was added and stirred constantly using sterile glass rod.

Required quantity of sweetner and stabilizer and flavoring agent was added.

Milk sample was cooked at 45°C and inoculated with 1 teaspoon of commercial yogurt or 10ml of culture.

The inoculated sample was wrapped and incubated at 45°C for 24 hrs.