

EXERCISE NO. 3

Date:

Aim of the Experiment:

STAINING AND MICROSCOPIC CHARACTERIZATION OF PROBIOTIC CANDIDATES

Introduction

Probiotic cultures are very important for the manufacture of good quality of fermented milk products. Contaminants may be from similar types of species or from other different groups of microorganisms that may come during handling or sub-culturing of cultures. Such contaminants create variety of problems to the starter performance. Therefore, dairy starter cultures should be periodically examined for contamination by extraneous organisms. The presence of these contaminants is highly objectionable as they bring about undesirable changes in the manufactured products. Microscopic examination of stained smear of cultures may give some preliminary idea about the contaminating organisms but conclusive detection of certain groups of organisms should be done by cultural methods.

(I) Grams Staining

Gram staining is the simple, inexpensive and quick test that gives idea about the type of shape (either coccus or rod) starter cultures exhibits during microscopic examination. It also indicates presence of other contaminants like yeasts, if present with the starter cultures as contaminant. The shape of starter cultures anything that found different than the expected are considered as contamination of starter. Such contaminated cultures should not be used for the manufacture of product.

Materials

1. *Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*
2. Glass slides
3. Wire or platinum loop
4. Microscope
5. Crystal violet or Methylene blue
6. Gram's iodine
7. 95% ethanol
8. Safranin
9. Cedar wood Oil
10. Xylene

Procedure

1. Take clean glass slides, wash it with detergent solution and allow it to dry

2. Prepare thin uniform smear of cultures to be examined.
3. Heat fixes the smears by moving glass slide (opposite side of the smear prepared) over the flame two to three times
4. Stain the smear with Crystal violet or Methylene blue for 1 min
5. Rinse the slide in tap water and apply Gram's iodine for 1 min.
6. Wash the slide and blot dry
7. Decolorize it with 95% ethanol for not more 30 sec.
8. Wash and blot dry.
9. Apply counter stain safranin for 1 min.
10. Wash and blot dry
11. Put a drop of cedarwood oil and examine the smear under oil immersion lens (100 X).
12. Record the observations, giving neat diagram.
13. Presence of any organism morphologically (shape) different from the starter organism indicates the contamination of the culture.

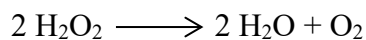
Note: Remove the cedar wood oil from the objective lens by Xylene and subsequently clean the Xylene with dry blowing paper.

(II) Catalase Test

All starter cultures are Catalase negative and therefore does not produce catalase enzyme, which may be used as basis for testing of purity of starter cultures. If the starter is contaminated by catalase producing organisms, the test results will show presence of gas bubbles in given condition.

Principle

When the microorganisms present in a culture produce catalase, it will produce air bubbles when the same is mixed with hydrogen peroxide.



Materials

1. Starter cultures (*Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp, *bulgaricus*)
2. Sterile test tubes (10 ml capacity)
3. Test tube stand
4. 10% H₂O₂ solution
5. Sterile pipettes

Procedure

1. Aseptically transfer 2.0 ml of the starter culture in a sterile test tube.

2. Add equal volume of 10% H₂O₂ solution and mix well.
3. Keep test tubes in a test tube stand and wait for few minutes
4. Observe for the formation of gas bubbles
5. Presence of effervescence indicates positive catalase test which is the indication of contamination of stater culture with non-lactic (catalase positive) organisms.
6. Use of such contaminated cultures for subsequent manufacture of fermented milk product should be avoided.

Observations

A) Grams Staining

Interpretation

B) Catalase Test

Interpretation