

EXERCISE NO. 2

Date:

Aim of the Experiment:

SAMPLING AND ISOLATION OF PROBIOTIC MICROORGANISMS

Introduction

Probiotics used by humans are usually isolated from different environments that include dairy and non-dairy sources. However, probiotics that are isolated from human or animal intestines have certain characteristics that differ from those isolated from dairy products. Isolation of *Lactobacillus* can be done from the milk sample by using selective media. *Lactobacillus* sp. was isolated from the milk samples by streak plate technique on MRS agar plates.

Materials

1. Samples for isolation (human breast milk, faecal samples, dairy products and fermented foods)
2. Inoculation needle
3. Incubator (set at 37°C)
4. Sterile test tubes (10 ml capacity)
5. Test tube stand
6. Sterile pipettes
7. Petri dishes

Procedure

A. Sampling

Collection and processing of faecal samples obtained from infants and adult

1. Faecal samples can be obtained from healthier adult volunteers and infants.
2. Samples were collected in a 5 mL sterile screw cap vial containing 0.1% peptone water.
3. After, bringing samples to laboratory nearly 1g of samples was transferred to 10 mL LAMBAV broth for enrichment containing 0.5 mg/mL LA.

Collection and processing of dahi and lassi samples

1. Samples of dahi and lassi were obtained from commercial dairy shops of nearby localities and also from traditional milk vendors.
2. Nearly, 1g of dahi and 1 mL of lassi was added to 0.1%(w/v) peptone water and mix by doing vigorous vortexing.
3. One millilitre of homogenized samples was added to 9 mL of LAMVAB broth for enrichment containing 0.5 mg/mL LA.

Collection and processing of mother breast milk samples

1. Mother's breast milk samples were collected with the help of trained nurse staff of the hospital in sterile 5 mL vials and transported immediately to laboratory in an ice box container.
2. Then 1 mL sample was enriched in 9.0 mL of LAMBAV broth containing 0.5 mg/mL LA.

Processing of Standard cultures

1. The received ampoules were aseptically broken, transferred to litmus chalk milk and then incubated at 37°C for 24-48 h. Next, these were transferred to MRS broth, incubated and checked for purity before employed in experiments.

B. Enumeration Procedure

1. Each sample was enriched at 37°C in modified MRS containing 0.5 mg/mL LA under micro-aerophilic conditions for 24 h.
2. After enrichment, 1.0 mL of sample was added to 9.0 mL of 0.1% (w/v) peptone water, vortexed and then serially diluted up to 10^{-4} - 10^{-10} dilutions.
3. To obtain colonies, 0.1 mL aliquot from appropriate dilutions was pour plated on MRS agar and further incubated at 37°C for 48 h.
4. Typical pin pointed yellow-colored colonies were randomly selected and transferred to MRS broth.
5. Further, cultures were checked for Gram and negative staining and thereafter, examined microscopically for preliminary purification and morphology.

Observations

A) Colony Morphology

Interpretation

B) Total microbial Load

Interpretation