Applied Biology and Bioengineering (BT520)

EXPERIMENT- 1

ASEPTIC CULTURE TRANSFER TECHNIQUE

PURPOSE

Technique of aseptic removal and transfer of microorganisms for sub-culturing.

PRINCIPLE

Microorganisms are transferred from one medium to another by subculturing. This technique is of basic importance and is used routinely in preparing and maintaining stock cultures, as well as in microbiological test procedures. The essential steps involved in aseptic transfer of microorganisms are as follows:

1. An inoculating loop is sterilized by holding it in the hottest portion of the Bunsen burner flame until the entire wire becomes red-hot. Once flamed, the loop is held in the hand and allowed to cool for 10 to 20 seconds.
2. The cotton plug of the stock culture tube is unplugged with the help of little finger, the neck of the tube is briefly passed through the flame and the sterile loop is further cooled by touching it to the sterile wall of the culture tube before removing a small sample of inoculum.
3. The neck of the stock culture tube is flamed and the cotton plug is reinserted.
4. The cotton plug of the subculture tube is unplugged likewise, the neck of the tube is briefly passed through the flame and the cell-laden loop is inserted into the subculture tube.
5. In case of a broth medium, the loop is shaken slightly to dislodge the organisms; with an agar slant medium, it is drawn lightly over the hardened surface in a zigzag line.
6. The neck of the subculture tube is flamed and the cotton plug is reinserted.
7. Following inoculation, the loop is again flamed to destroy remaining organisms.
8. In case of liquid-liquid transfer, sterile tips are used to inoculate cultures (1%).

MATERIALS

Cultures

1) 12-h nutrient broth culture of *E. coli* 2) 24-h nutrient-agar slant of *E. coli*

MEDIA COMPOSITION

I. NUTRIENT BROTH

Ingredients	**gl^{-1}**
Peptic digest of animal tissue	5.00
Yeast Extract	1.50
Beef extract	1.50
Sodium chloride	5.00
Final pH (at 25℃) 7.4 ± 0.2	

II. NUTRIENT AGAR

Ingredients	**gl^{-1}**
Peptic digest of animal tissue	5.00
Yeast Extract	1.50
Beef extract	1.50
Sodium chloride	5.00
Agar	15.00
Final pH (at 25℃) 7.4 ± 0.2	

PREPARATION

I. NUTRIENT BROTH

The ingredients were dissolved in distilled water, dispensed as 5.0 ml aliquots in test tubes (18 x 150 mm), plugged with cotton and autoclaved at 121℃ for 20 min.

II. NUTRIENT AGAR

Nutrient agar slants were prepared by adding requisite amounts of the media ingredient to distilled water. The agar was dissolved by heating in a microwave oven. The loss of water due to evaporation was compensated and the medium was distributed as 10 ml aliquots in test tubes (18 x 150 mm), plugged with cotton and autoclaved at 121℃ for 20 min. After sterilization, the media was allowed to cool down to about 40-45℃, and the tube was then slanted on the working bench

EQUIPMENT

Laminar hood, inoculating loop, glassware marker, sterile disposable tips, micropipette

PROCEDURE

Following the procedure previously outlined, the following transfers were performed:

1. *E.coli* broth culture to nutrient broth
2. *E.coli* slant and broth culture to nutrient broth and nutrient agar slant.
3. The broth cultures were incubated in a shaker incubator set at 37℃ and 180 rpm for 24h
4. The slant cultures were incubated in an incubator set at 37℃ for 24 h

OBSERVATIONS AND RESULTS

Culture	Growth	Contamination
Nutrient broth (Liquid-liquid transfer)		
Nutrient broth (Solid-liquid transfer)		
Nutrient agar slant (Liquid-solid transfer)		

Indicate growth as '+' and no growth/no contamination as '-'

REVIEW QUESTIONS

1. Explain why the following steps are essential during subculturing:
    a. Flaming the inoculating loop prior to and after each inoculation
    b. Cooling the inoculating loop prior to obtaining the inoculum
    c. Flaming the neck of the tubes immediately after unplugging and before replugging

2. Cite the purpose of the subculturing procedure?
3. Upon observation of your subculture broth and agar slant you do not observe any bacterial growth. What could be the possible reasons for the same?