LAB: 4 principle of biotechnology

Maintaining and preserving pure cultures

Once microorganism has been isolated and grown in pure culture, it is necessary to maintain the viable culture; free from contamination, for some period of time. There are several methods available for maintaining and preserving pure cultures include:

1- Subculturing:

The organisms may simply be subcultured periodically into a fresh medium to permit continued growth and to ensure the viability of a stock culture. Aerobes are maintained on agar slants. Anaerobes are maintained by growing the bacteria deep in the agar where air is excluded; this is achieved by stabbing an agar tube with an inoculating needle coated with a bacterial inoculum to produce a stab culture. For more stringent anaerobic conditions, cultures can be covered with 2-3cm of sterile mineral oil, and incubated in anaerobic chambers.

Unfortunately, frequent subculturing introduces high risk, since some genetic and physiological changes will occurred within the strain. Additionally; it is time consuming method, especially if large numbers of cultures are involved.

2-Maintenance at low temperature by refrigeration:

Longer storage times can be achieved by lowering the temperature conditions for storage. Under these conditions, bacterial metabolism is sharply reduced and cultures can be maintained for 3-5 months at refrigerator temperatures 4-5 C˚.

3- Freezing:

Much longer storage times are possible when cultures are stored at -196 to -20 c˚. These temperature requirements can be achieved by commercial ultra cold freezers or by storing cultures in containers of liquid nitrogen. The rapid freezing of cells is obligatory, as freezing induces ice crystal formation in cells that can lead to mechanical lyses and cell death, (often, protecting material such as glycerol is added to the culture). Glycerol is often employed as an antifreeze agent to prevent damage due to ice crystal and to ensure the ability to recover viable microorganisms when frozen cultures are thawed.

4-Drying:

Removal of water also reduces rates of microbial metabolism, producing non metabolizing cultures that are not subjected to genetic or physiological changes. This method is particularly used to endospores forming bacteria. Endospores do not carry out active metabolism and are relatively dry. After drying process, the cultures must be covered to prevent air entrance.

In these types of cultures we use soil, sand, silica gel as carrier. They have been known to remain viable for centuries. The procedure involve mixing of 20% soil ,78% sand and 2% calcium carbonate then sterilized in oven temperature 130 C˚ for 8-10 hours ,after cooling inoculate the tube with dried spore suspension and then store at room temperature.

5- Lyophilization:

Simple desiccation of non-endospore forming bacteria is rarely used because of the loss of viability of most active bacterial cells during the drying process. Desiccation for long -term preservation of most cultures can be achieved by freeze-drying or lyophilization.cell suspensions, usually in a medium containing a protecting substance, are quick frozen in a dry ice acetone bath. They are then desiccated in the frozen state using a high vacuum to sublime the water directly

from the solid to the gaseous form. Cultures can be stored for many years without any appreciable loss of viability.

Maintaining stock cultures by subculturing

Materials:

Petri plate with well isolated colonies

Nutrient agar slant

Inoculating loop

Bunsen burner

Procedures:

1-Using aseptic technique pick a well isolated colony and, using the inoculating loop that you have sterilized in a Bunsen burner flame, Transfer some of the cells to each of two labeled agar slants.

2- Place inoculated tubes into a 37 c˚ incubator for 24-48 hours.

3- At the next laboratory transfer one of the tubes to a refrigerator and the other to a room temperature or 28 c˚incubator

4- Store the cultures for six weeks.

5- After storage compare the appearances of the culture stored at 25-28 c˚ and the culture stored at 5 C˚. Aseptically transfer material from each culture tube to fresh nutrient agar slants.

6-Incubate the inoculated tubes at 37 c˚for 24-48 hours.

7- At the next laboratory session observe the new slants and describe their appearances.