biotechnology

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Safety measures

In microbiology laboratory you deal with different microorganisms some of them may be pathogenic. For your safety, certain rules and instructions must be employed including:

1- Every microorganism used in the laboratory must be treated as a pathogenic one.

2- Do not take any living culture out of the laboratory.

3- Surface disinfection of your bench before and after each laboratory session.
4- Report any accident or spills to your instructor and spills are covered with paper towels and disinfected for at least 15 minutes.

5- All materials contaminated with microorganisms must be placed in a specified container to be sterilized.

6- Don’t place handbag, book bag, or similar belongings on the floor.

7- Do not eat or drink in laboratory and keep hands, pencils, and similar things away from your mouth.
8- Don’t place unnecessary materials on your workbench.

9- Wash your hands with worm soapy water or disinfectant solution before leaving the laboratory.

10- Wear a laboratory coat.

11- Tie long hair back and up. Also a scarf, cap, headband, or other covering is used for fluffy or flyaway hair.

12- Revise the assigned exercise(s) before the laboratory session.
Biotechnology

a) Background

Biotechnology is best defined as the exploitation or application of biological systems (microbial, animal or plant cells or enzymes) to gain a useful product or service.
Many scientific disciplines contribute to molecular biotechnology, generating a wide range of commercial products.
b) Recent development and approaches:

The recent milestone development in biotechnology that renders this science at the frontier of science is tremendous developments in other areas of science including:

1- Biocatalysis
2- Immunology
3- Genetic engineering
4- Fermentation technology
• Categories of products:

1- Biomass: The product may be the cells themselves e.g. baker's yeast or single cell protein (SCP).

2- Enzyme: e.g, amylase, penicillin acylase

3- Metabolites: either primary metabolite such as citric acid which are usually produced during the logarithmic growth phase (trophophase) or secondary metabolites such as antibiotics, alkaloids or glycosides which are produced during the stationary phase (idiophase).

4- Biotransformation product: e.g. steroid transformation.
5- Biodegradation product: degradation of xenobiotics (insecticides and petroleum oil)
6- Immunological product: vaccines and monoclonal antibodies (MCA).
7- Energy: alcohol, methane (biogas).
8- Genetically engineered therapeutic protein: Insulin, growth hormone and interferon.
9- Intra or extracellular accumulation of metals.
10- Plant tissue culture: cell suspension, callus and hairy root.
Fermentation technology

- Fermentation is an industrial process utilizing living cells for the production of commercially valuable products either aerobically or anaerobically. In fermentation, living cells are allowed to grow under defined conditions in a fermenter (bioreactor). The defined conditions include the use of the proper substrate (medium) and the proper environmental parameters (e.g. temperature, agitation, pH and aeration). All must be optimized to achieve the highest yield and highest quality at the lowest cost possible.
1-Microbial culture

Source:

1- isolation of m. o

How to isolate organisms from soil water sludge

- Enrichment culture technique

- Medium used

- Technique

- 2-Culture collection: American Typing Culture Collection (ATCC) and National Collection of industrial bacteria (NCIB).

Characters of organisms to be used in biotechnology
. Improvement of product quality and quantity by is carried out by manipulating the process, including:

1. **Upstream manipulation** (Strain development) The productivity of the wild strains are usually too low for economical processes and so several years of extensive research programs may be required to develop a strain that could be used for large-scale production. Such programs include
i) Selection of the biological entities by a screening program to choose the best biocatalyst of the required product.

ii) Utilization of mutation techniques to prepare mutants of better characters.

iii) Utilization of recombinant DNA (genetic engineering) to improve an existing process or developing a totally new product.

iv) Cell fusion for the generation of hybrids with improved productivity.

v) Plant cell and tissue culture
II- **Downstream** processing: It includes manipulation of the fermentation conditions to specify the product and maximize the yield e.g. changing the oxygen potential of growth for Saccharomyces yields different product:

- Aerobic Saccharomyces + Hexoses ➤ Baker's yeast
- Anaerobic Saccharomyces + Hexoses ➤ Alcohol
- Anaerobic Saccharomyces + Hexoses & bisulphite ➤ Glycerol
Bioreactors:

Vary from very simple vessel with few controls to highly complicated ones in which the whole process is computerized. In aerobic bioreactors, maximum optimal aeration and good agitation are applied, while in anaerobic fermentation no aeration and minimal agitation are used.
Autoclavable bench-top laboratory bioreactor used for fermentation and cell cultures
Tower fermentor
Isolation of pure bacterial cultures

- "A pure culture is defined as a population of cells which arose from a single cell by repeated cell division...”.
- Used to separate numerous bacteria, in a tested material, one from other
- based on *mechanical or biological separation* of bacteria:
Mechanical separation method:

- Based on mechanical dispersion of microorganisms by serial dilution in liquid nutrient media through:
  1. Fractional dilutions technique.
  2. Pour plate technique (Dilution in solid nutrient media)
  3. Spread plate technique (Superficial dispersions)
  4. Streak plate technique
Serial dilution
Isolation of soil microorganism

Procedure:

1- Collect soil samples from different locations.

2- Soil is dried about one week at room temperature.

3- One gram of each dried soil sample, placed in a sterile beaker, cup, or other containers, is suspended in 9 ml sterile saline. Mix well and leave the soil particles to settle.

4- One ml, from the supernatant, is 10 fold serially diluted using set of 9 ml of sterile saline tubes as follow:
Label sterile test tubes (1 to 6).

Aseptically distribute 9 ml of sterile saline into each tube with 10 ml pipette.

Add 1 ml soil supernatant into the first tube. Mix and transfer 1 ml from tube 1 to tube 2. Repeat until tube No. 6 using 1 ml pipette.

The dilutions in the tubes no. 2-6 will be $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, and $10^{-6}$.

A total volume of 0.1 ml from the $10^{-3}$ through $10^{-6}$ dilutions will be used to obtain isolated colonies on agar plates for further studies, using one of the following isolation techniques:

1. The spread plate technique.
2. The pour plate technique.
3. The streak plate technique.
1. The spread plate technique:

Procedure:

- Four agar plates are labeled from $10^{-3}$ to $10^{-6}$.
- Transfer 0.1 ml, from each soil dilution ($10^{-3}$ to $10^{-6}$ dilutions) on the agar surface in agar plates.
- Sterilize glass spreader by placing in alcohol and flaming. When the fire is off, spread the inoculum all over the surface of agar.
- After incubation in an incubator over a defined time period, usually between 12 and 48 hours, the surface-dispersed cells grow into distinctive and visible colonies.
2. The pour plate method:
3. The streak plate technique:
These plates are good examples of streak plate technique. Isolated colonies are visible. Plate B shows three types of colonies, the other plate shows a pure culture.
Notes: The large, glassy, translucent colonies are bacteria. The fuzzy colonies are filamentous fungi. The small white opalescent colonies are yeasts (unicellular fungi).
Below is shown the appearance of a *Streptomyces* isolation plate after 5 days of incubation.
Identification and Examination of soil micro-organisms

- **Materials**
  - 1-2 plates transfer media
  - wire loop
  - Bunsen burner

- **Procedure**

*Streptomyces* species are the white and colorful chalky looking colonies. The following picture is a closeup of a typical *streptomyces* colony.
Examine the PDA plates and look for typical *Streptomyces* colonies. They are small, opaque, compact, frequently pigmented (brown, yellow, pink, etc.), often leathery, and appear dry and dull looking. Typically, a depression in the agar surface will be observed around the colony. Avoid molds. They usually form much softer, fuzzy colonies if present.

Prepare a wet mount. Good *Streptomyces* candidates will be difficult to remove from the agar with the inoculating needle or loop and upon observation under the microscope will reveal a multitude of spores with a few filamentous cells.

With the inoculating loop Streak *Streptomyces* colony on the transfer media for isolation of pure colonies.

Incubate for 3-5 days at room temp (at dark)
Note the colorful chalky/dusty appearance. The colony is hard, not gummy, and does not easily lift from the agar.
Description

- *Streptomyces* are gram positive, spore-forming bacteria found in soil. They are characterized by their tough, leathery, frequently pigmented colonies and their filamentous growth. *Streptomyces* are chemoheteroorganotrophs, growing best at 25°C and pH 8-9. They are capable of using complex organic materials as carbon and energy sources and are involved in the breakdown of these products in the soil. This degradative ability makes these bacteria pivotal in the production of fertile soil for agriculture. They also give soil its characteristic smell by the production of a class of volatile low molecular weight compounds called geosmins.
In the laboratory, different isolates were found to produce numerous compounds capable of inhibiting or killing other microorganisms. *Streptomyces* are also of medical and industrial importance because they synthesize antibiotics. There are several theories to may explain antibiotic production; the most widely accepted one being that antibiotics help the organism compete with other organisms in the relatively nutrient-depleted environment of the soil by reducing competition. Over 50 different antibiotics have been isolated from *Streptomyces* species, including *streptomycin*, *neomycin*, *chloramphenicol* and *tetracyclines*. 
Results:

Source.

Total number of colonies.

Number of different colonies.

Description of colonies:

1- Shape (circular-irregular)

2- Size (small- medium- large)

3- Color
4- Margin (entire- irregular)

5- Texture (soft- hard- mucoid)

6- Optical characters (transparent- opaque)

7- Elevation (flat- convex- raised)

8- Surface (smooth, rough, shiny)