Antibiotic Production:

- In 1929, Alexander Fleming discovered penicillin almost accidentally , and the medical use came during the 1939-1945.
- **Antibiotic:** This term was used to describe substances produced by microorganisms that could be used to kill or inhibit growth of certain other microbes .
- **Antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.
- **Antibiotics :** are the secondary metabolites of microorganisms.
- Secondary metabolites (SMs) are natural products synthesized mainly by bacteria, fungi and plants. They are molecules of low molecular weight with diverse chemical structures and biological activities. The name secondary metabolite originates from the initial observation that their production is not necessary for the growth and reproduction of organisms, in contrast to primary metabolites which include lipids, amino acids, carbohydrates and nucleic acids. The synthesis of secondary metabolites is very dependent on the culture conditions, particularly the composition of the medium.
- Similar techniques for the large scale culture of microorganisms can be used for the production of enzymes, such as **a-amylase** by the bacterium ***Bacillus licheniformis*.** Many enzymes used in industry are extracellular and are excreted by the microorganisms into the culture medium.

- Extracellular enzymes can be extracted from the medium by a process of filtration, to remove the microorganisms, then reverse osmosis is used to separate the enzyme from other components of the medium. The extraction of intracellular enzymes is more complex and involves cell disruption, followed by purification of the enzyme. Cells are disrupted to release the enzymes, by treatment with detergents, or lysozyme (an enzyme which digests some bacterial cell walls), or by mechanical methods. After removal of cell debris, the enzyme may be purified and concentrated using, for example, ammonium sulphate solution which will precipitate the enzyme from solution.
- a few microbial organisms produce the majority of secondary metabolites and a single microbial type has the capacity to produce very different metabolites, for example, *Streptomyces griseus* and *Bacillus subtilis* each can produce more than 50 different secondary metabolites. The production of economically valuable secondary metabolites (e.g., antibiotics) is one of the major activities of the bioprocess industry. The most common secondary metabolites are antibiotics; others include mycotoxins, ergot alkaloids, the widely used immunosuppressant cyclosporin, and fumagillin, an inhibitor of angiogenesis and a suppressor of tumor growth

- Antibiotics are produced industrially by a process of fermentation, where the source microorganism is grown in large containers (100,000 – 150,000 liters or more) containing a liquid growth medium.
- In order to maximize the production of penicillin, nutrients such as nitrogen sources may be added to the medium towards the end of the growth phase this is referred to as **fed-batch culture**.

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- Microbicidal: The substances were used to kill the other microbes.

- Microbistatic: The substances were used to inhibit or retard growth of microorganisms.

- The antibiotic action depend on :

a) The concentration of antibiotics.

b) Type of bacteria: Gram positive (G+) bacteria are more sensitive to antibiotics than Gram negative (G-) bacteria .

The effectiveness of an antibiotic is described as :

- Broad spectrum : when it acts on a wide range of (G+ve) and (G-ve) bacteria
- Narrow – spectrum antibiotics: are more specific , these can be useful medically because they target a limited range of microbes .

The mechanism of action of antibiotics :

1) Interference with cell – wall synthesis (in bacteria) .
2) Interference with membrane function (in fungi).
3) Protein synthesis.
4) Nucleic acid synthesis.

Commercial Sources:

Penicillium notatum, Penicillium chrysogenum .

Antibiotic resistance:

can be defined as the acquired ability of microorganisms to resist the effects of an antibiotic to which it is normally susceptible .

Some organisms are naturally resistant , whereas others may developed and acquired by genetic mutation (conjugation) transduction or transformation in bacteria .

Microbes for Biological Control:

Microbial pathogens can thus be exploited as biological control agents, and success has been achieved using bacteria, fungi and viruses. The term microbial insecticide is used when the microorganism is used to control insects, and the term mycoherbicide when pathogenic fungi arc used to control weeds. While only a few mycoherbicides are currently being used commercially, it is an area where further research may be successful in providing alternatives to chemical herbicides for weed control.

A well established example of a microbial insecticide is *Bacillus thuringiensis.* This bacterium produces a glycoprotein, known as Bt, which is toxic to a variety of insects, such as butterflies ,moths and beetles, but not to animals and humans. When ingested by the insect larvae, the toxin leads to paralysis or degeneration of the gut. The insect is usually killed within a few hours.

Plant and animal cell culture

The principles involved in the culture of microorganisms can be applied to the culture of cells and tissues obtained from plants and animals. ‘Essentially, this involves the culture of suitable cells under aseptic conditions, in complex media which have been specially formulated for this purpose. The maintenance of strict aseptic conditions is essential in cell and tissue culture, as any contaminating microorganisms are likely to grow very much faster than the plant or animal tissue.

Plant tissue culture involves the growth of isolated cells or tissues in controlled, aseptic conditions. It is possible to use plant tissue culture to

regenerate whole plants, a technique referred to as micropropagation. One of the uses of this technique is to propagate rare, or endangered, species which are difficult to propagate using conventional methods of plant breeding. Micropropagation is also used to produce ornamental plants, including pot plants, cut flowers and orchids on a large scale for commercial purposes. The techniques of plant tissue culture are also used to eliminate pathogens from infected plants, for example in the production of virus-free plants, such as carnations and potatoes. There are a number of different types of plant tissue culture, including:

- Embryo culture, cultures of isolated plant embryos
- Organ cultures, cultures of isolated organs including root tips, stem tips, leaf buds and immature fruits
- Callus cultures, which arise from the disorganized growth of cells derived from segments of plant organs, such as roots.

The isolated part of the plant used for culture is referred to as the explant, which can be almost any part of the plant. The tissue used as the explant is grown in culture media containing a variety of mineral nutrients, plant growth regulators such as auxins and cytokinin's, sucrose, and amino acids.

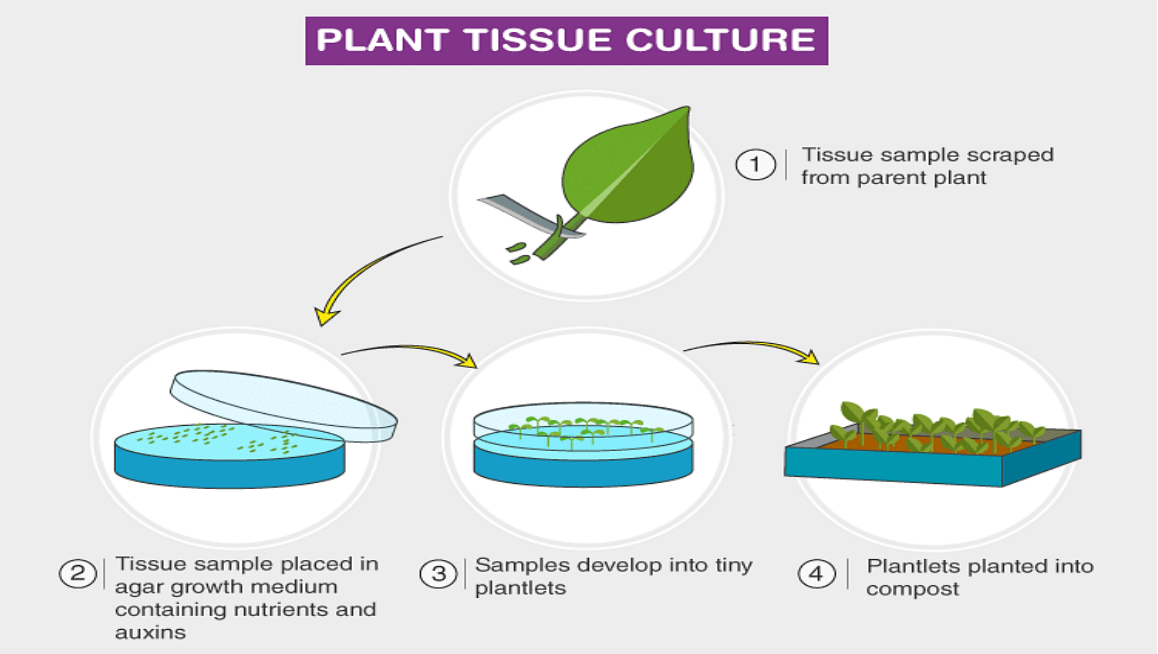
A callus culture may be grown by removing tissue from a suitable plant organ such as a carrot. This must be surface sterilized by placing it in a suitable chemical disinfectant such as 20 per cent sodium hypochlorite solution. The carrot is then washed with sterile distilled water and, using sterile instruments and aseptic technique, a segment of tissue removed from the cambium. This is then transferred to a flask containing sterile culture medium and incubated at 25 °C. The explant will grow to form a mass of pells known as a callus, which has a distinctive crumbly appearance. The callus can be maintained indefinitely by sub-culturing

the tissue onto fresh medium every 4 to 6 weeks, or the callus can be transferred to a medium containing a different balance of plant growth regulators and can be. induced to form structures known as embryoids, from which complete, plants can be regenerated. This method has a number of important commercial applications, such as the rapid propagation of agricultural crop plants.

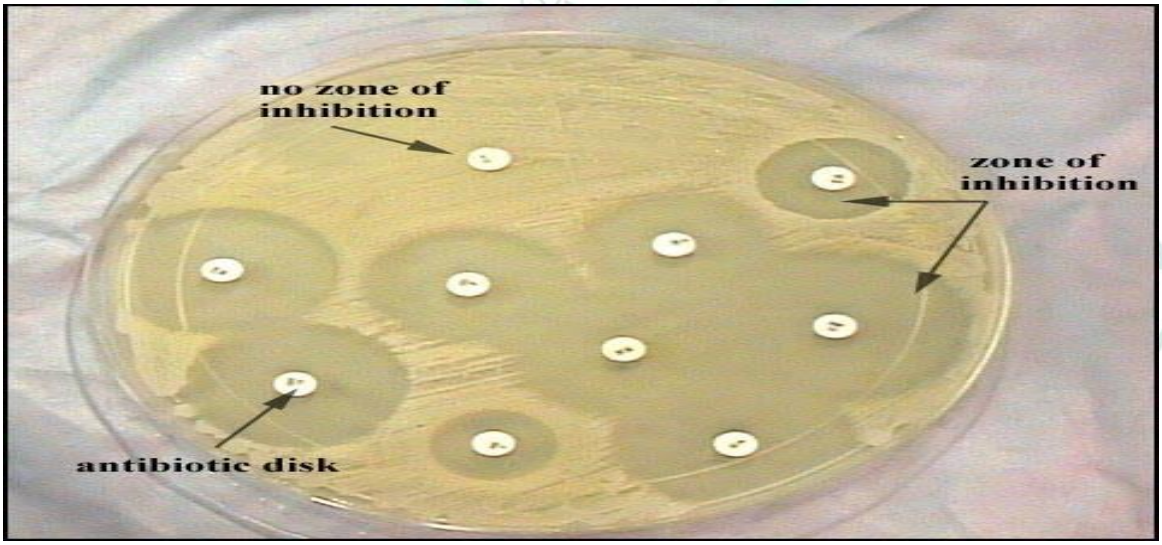
Animal cells which are cultured can be derived from explants of the four basic tissue types, epithelial, connective, nervous or muscular tissues. Some .of these cells, such as lymphocytes (derived from connective tissue), can be grown in a suspension culture, similar to bacteria in a liquid medium. Most normal mammalian cells, however, grow attached to a surface and form a single layer of cells referred to as a monolayer. Tissues removed from an animal are usually treated with a proteolytic enzyme, such as trypsin, to separate individual cells. The cells are then washed in sterile saline solutions and transferred to a suitable sterile container, such as a plastic flask, containing a culture medium.

The cells settle on the bottom of the flask, attach, and begin to divide to form a monolayer. The cells can be removed, by treatment with trypsin, and used to inoculate fresh medium. In this way, the growth of some cells can be maintained indefinitely, whereas some cells have a finite capacity for growth.

Media used for animal cell culture are usually very complex and contain a range of amino acids, glucose, vitamins and other enzyme cofactors, inorganic ions and buffers to maintain the pH. Serum may also be added to the media to provide essential growth factors. Antibiotics, such as penicillin and streptomycin, are sometimes added to the media to inhibit the growth of bacteria which may accidentally contaminate the cultures.



Plant tissue culture on a sterile agar medium



An antibiotic sensitivity test