# Antibiotics:

## Production of Antibiotics: Penicillin and Streptomycin :

Penicillin was the first antibiotic discovered by a classical observation of the inhibition of *Staphylococcus aureus* growth on agar plates due to a contaminating mold later identified as *Penicillium notatum* by Alexander Fleming in 1929. The demand of chemotherapeutic agents for preventing the bacterial infection encountered by the soldiers in World War II led to the development of industrial scale production of penicillin. At that time many of the bacterial infections were fatal since no antibiotics were available for their prevention. Antibiotics are the secondary metabolites produced by one organism which inhibits the growth of other organism even at a very small concentration. Till date a huge number of antibiotics have been discovered mainly produced by fungi, bacteria, and actinomycetes. Actinomycetes produce largest number of antibiotics of various structures, especially the genus *Streptomyces*.(p 31,32).

## Mechanism of action:

Penicillin inhibits the cell wall synthesis in growing bacterial cells by combining with the so-called penicillin-binding protein (PBP) of the bacterial cell wall involved in the transpeptidation reaction (cross linking) of the peptide side chains of the adjacent peptidoglycan in the bacterial cell wall. Due to the defective cell wall, the bacteria are unable to withstand the osmotic shocks and are ultimately get lysed.

## Strains and production of penicillin:

The strain of *Penicillium notatum* discovered by Fleming had low productivity (2 IU/ml). Later in the year 1945 Raper and Alexander found a new strain *Penicillium chrysogenum* having higher yield of penicillin. Further improvement was achieved by inducing mutations via UV irridaition and strain Wis Q 176 was isolated. Presently the strains have been improved much to produce 15000 IU/ml of penicillin during fermentation.

The media used for the production of penicillin contains cornsteep liquor solids 3.5%, lactose 3.5%, glucose 1%, calcium carbonate 1%, potassium dihydrogen phosphate 0.4%, edible oil 0.25% and penicillin precursors. Submerged fermentation processes in 40,000-200,000 liter fermenters with high aeration and agitation is carried out. The pH of the media is maintained around 5.5-6.0 and temperature about 25-27 ºC. Lyophilized spores are used as inoculum to a concentration of $5 \times 10^3$/ml medium and development of loose mycelial pellets are required for better production of penicillin. The penicillin fermentation process can be divided into two phases i.e. first is the **growth phase** and the second is the **penicillin production phase**. The growth phase lasts for about 40 h during which the cell mass increases very rapidly (doubling time 6 h) and oxygen demand is very high. After this phase the penicillin production phase starts in which the biomas production is greatly reduced (specific growth rate, $\mu = 0.01$) and rate of penicillin production increases. Various media component are fed during this phase to extend the penicillin production for 120-180 h.

## Recovery of penicillin :

In the downstream processing of penicillin G, the fermentation broth is first chilled and passed through rotary filter to remove biomass. The filtrate is then acidified and the penicillin is extracted with butyl acetate. Penicillin is extracted from solvent (butyl

acetate) into aqueous buffer with pH 7.0. The aqueous fraction is acidified to pH 2.0-2.5 with $H_2SO_4$ and re-extracted into butyl acetate. Potassium acetate is added to the extract which causes the formation of penicillin $GK^+$ salt crystals via a number of back-extraction and crystallization steps. The salt so formed is washed with acetone by centrifugation and dried under vaccum.

## Streptomycin:

Streptomycin is a broad spectrum antibiotic belonging to oligosaccharide antibiotic/aminoglycoside family. Streptomycin was discovered by Schatz, Bugie, and Waksman in 1944 from *Streptomyces griseus* isolated from soil. This antibiotic is mainly active against Gram negative bacteria, *Mycobacterium tuberculosis* (causing agent of tuberculosis) and also inhibits the growth of some gram positive bacteria and is mainly used against the pathogenic bacteria resistant of penicillins. Its major use in the anti-TB drug formulations and is available as sulphate salt. This antibiotic is also active against other diseases, e.g., plague (*Pasteurella pestis*), brucellosis (*Brucella abortus*), tularemia (*Francicella tularieusis*). This antibiotic interferes with the functioning of $7^{th}$ cranial nerve and can also cause deafness and kidney damage. Streptomycin is also used in the treatment of plant diseases since this antibiotic is systemic (i.e. transported to all parts of the plant through vascular tissue). Streptomycin has three constituents namely; N-methyl L-glucosamine, Streptose and streptidine .

## Mechanism of action:

In sensitive organism the streptomycin binds to the S12 protein of the small subunit of ribosome, which is also the m-RNA binding site during translation. There is competition between the streptomycin and m-RNA for the binding site and thus this antibiotic hinders with the formation of initiation complex as a result the fidelity of the genetic code is not maintained. There is misreading of the genetic code and defective proteins are synthesizing resulting in the death of the microbe.

## Production of streptomycin:

The present day streptomycin producers are the mutants of *Streptomyces after* screened extensively for higher yields. In the beginning of 1940s, *Streptomyces* strains were poor yielding and only 50 units of streptomycin per ml of broth were produced. New strains developed by mutation and selection yield 25,000 units per ml.

The industrial production of streptomycin is carried out using submerged fermentation processes. The spores of *Streptomyces* mutants are maintained as soil stock or lyophilized form since these strains are genetically unstable. The spores from the stock are used for inoculating sporulation medium, which is then transferred to germinator where biomass is increased for inoculating fermenters. Media consisting of glucose, starch, dextrin, soy meal, corn steep liquor, sodium sulphate are used for the fermentation. The streptomycin fermentation requires high aeration and agitation. The fermentation is carried out at 28-30 ºC and medium pH 7.6-8 for good productivity. The fermentation lasts for 5-7 days with an yield of 1-3 g/L of the fermentation broth.

The streptomycin fermentation proceeds through three phases. In first phase that lasts for 24 h the organism produces proteases which digest the soybean meal releasing ammonia and carbohydrates to be used for increasing the biomass. Glucose utilization is slow and low count ratio of streptomycin is produced during this phase. The medium pH also rises from 6.7 or 6.8 to 7.5 or higher. The next phase is the streptomycin producing phase which ranges from 24 h to 6-7 days. Rapid utilization of ammonia and glucose occurs and substantial production of streptomycin is observed. There is no mycelial growth and pH during this phase remains fairly constant around 7.6 to 8.0. This is the idiophase or the stationary phase during which the streptomycin (secondary metabolite) is produced. In the last phase (death phase) the sugars have been completely depleted in the medium and streptomycin production ceases completely. The ammonia released due to the cell lysis raises medium pH. Fermentation broth is generally harvested before the last phase begins.

## Recovery of streptomycin :

On completion of fermentation, the mycelium is separated from the broth by filtration and streptomycin is recovered by passing through ion exchange columns where it gets adsorbed. Streptomycin is then eluted from the column as streptomycin sulphate. Further impurities are removed by treating it with sodium hypochlorite, EDTA and activated carbon. The purified streptomycin sulphate solution is concentrated under vacuum and dried aseptically. The ion exchange resin columns have replaced the traditional solvent extraction methods employed for the extraction and purification of streptomycin which significantly reduced the cost of streptomycin production.