PRODUCTION OF SOME IMPORTANT SECONDARY METABOLOITES

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all.

Penicillins:

Penicillins are a group of β-lactam containing bactericidal antibiotics. Being the first among the antibiotics to be discovered, penicillins are historically important. The structures of important synthetic and semi-synthetic penicillins are depicted in Fig. 25.1. The basic structure of all the penicillins consists of a lactam ring and a thizolidine ring fused together to form 6-aminopenicillanic acid.

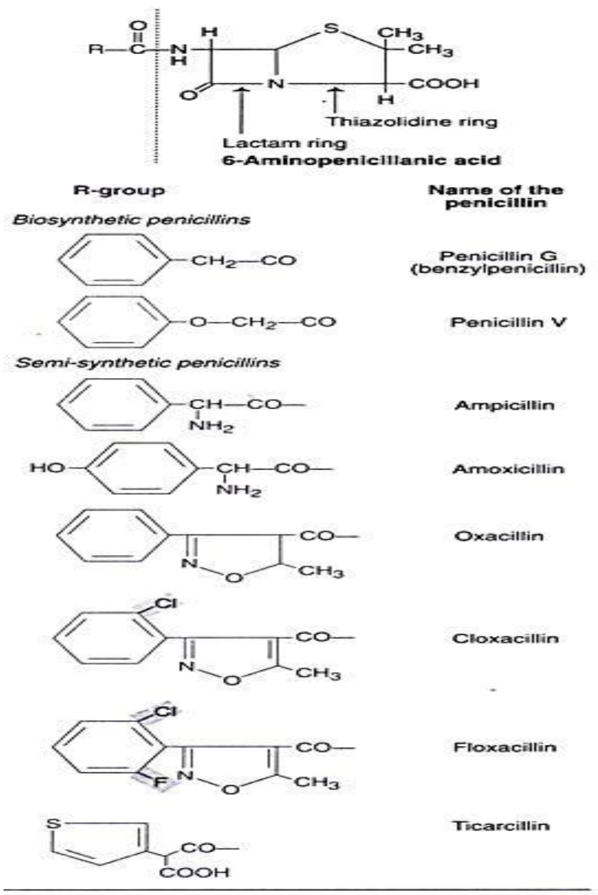

Fig. 25.1 : Structures of important penicillins.

Action of Penicillins:

Natural penicillins (penicillins V and G) are effective against several Cram-positive bacteria. They inhibit the bacterial cell wall (i.e. peptoglycan) synthesis and cause cell death. Some persons (approximately 0.5-2% of population) are allergic to penicillin.

Natural penicillins are ineffective against microorganisms that produce β-lactamase, since this enzyme can hydrolyse penicillins e.g. Staphylococcus aureus. Several semi-synthetic penicillins that are resistant to β-lactamase have been developed and successfully used against a large number of Gram-negative bacteria.

Cloxacillin, ampicillin, floxacillin and azlocillin are some examples of semi-synthetic penicillins. These are quite comparable in action to cephalosporin's. From the huge quantities of penicillins produced by fermentation, about 40% are used for human healthcare, 15% for animal healthcare and 45% for the preparation of semi-synthetic penicillins.

Organisms for Penicillin Production:

In the early days, Penlcillium notatum was used for the large-scale production of penicillins. Currently, Penicillium chrysogenum and its improved mutant strains are preferred. Previously, the penicillin production used to be less than 2 units/ml, and with the new strains, the production runs into several thousands of units/ml. One of the high yielding strains wis Q176 is preferred by several penicillin manufacturers.

Genetic engineering for improved penicillin production:

Some of the genes involved in penicillin biosynthesis by P. chrysogenum have been identified. Genetic manipulations were carried out so as to substantially increase the penicillin production. For instance, extra genes coding for the enzymes cyclase and acyltransferase have been inserted into C. chrysogenum.

Biosynthesis of Penicillin:

L-α-Aminoadipic acid combines with L-cysteine, and then with L-valine to form a tripeptide namely α-L-aminoadipylcysteinylvaline. This compound undergoes cyclization to form isopenicillin which reacts with phenyl acetyl CoA (catalysed by the enzyme acyltransferase) to produce penicillin G (benzyl penicillin). In this reaction, aminoadipic acid gets exchanged with phenylacetic acid (Fig. 25.2).

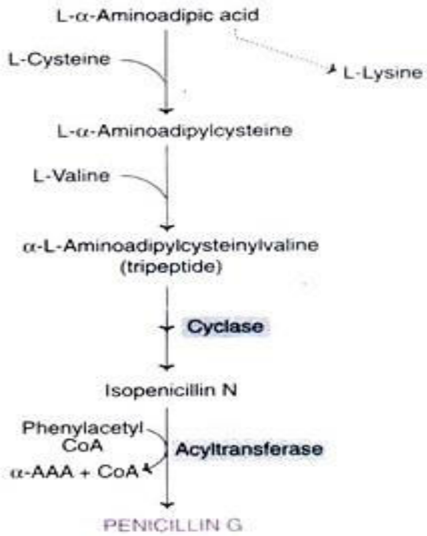

Fig. 25.2 : Biosynthesis of penicillin by Penicillium chrysogenum *(α-AAA — α-Amino adipic acid; CoA–Coenzyme A.*

Regulation of biosynthesis:

Some of the biochemical reactions for the synthesis of penicillin and lysine are common. Thus, L-α-aminoadipic acid is a common intermediate for the synthesis of penicillin and lysine. The availability of aminoadipic acid plays a significant role in regulating the synthesis of penicillin.

Penicillin biosynthesis is inhibited by glucose through catabolite repression. For this reason, penicillin was produced by a slowly degraded sugar like lactose. The concentrations of phosphate and ammonia also influence penicillin synthesis.

Production Process of Penicillin:

An outline of the flow chart for the industrial production of penicillin is depicted in Fig. 25.3. The lyophilized culture of spores is cultivated for inoculum development which is transferred to pre-fermenter, and then to fermenter.

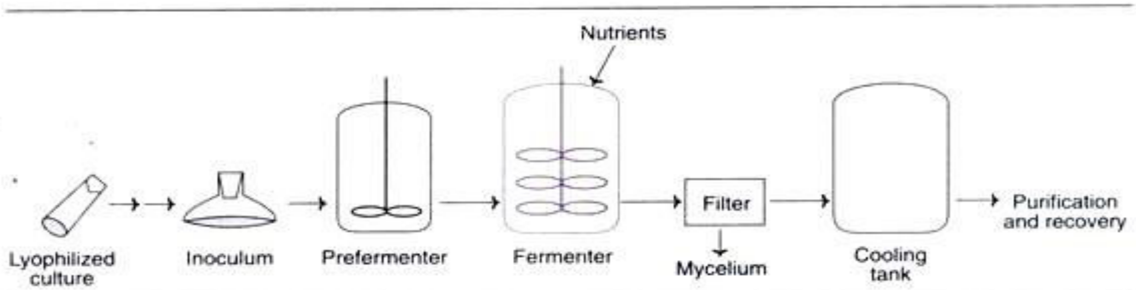

Fig. 25.3 : An outline of the flow chart for penicillin fermentation.

Penicillin production is an aerobic process and therefore, a continuous supply of O_2 to the growing culture is very essential. The required aeration rate is 0.5-1.0 vvm. The pH is maintained around 6.5, and the optimal temperature is in the range of 25-27°C. Penicillin production is usually carried out by submerged processes. The medium used for fermentation consists of corn steep liquor (4-5% dry weight) and carbon source (usually lactose). An addition of yeast extract, soy meal or whey is done for a good supply of nitrogen.

Sometimes, ammonium sulfate is added for the supply of nitrogen. Phenylacetic acid (or phenoxyacetic acid) which serves as a precursor for penicillin biosynthesis is continuously fed. Further, continuous feeding of sugar is advantageous for a good yield of penicillin. The penicillin production profiles are depicted in Figs. 25.4 and Fig. 25.5.

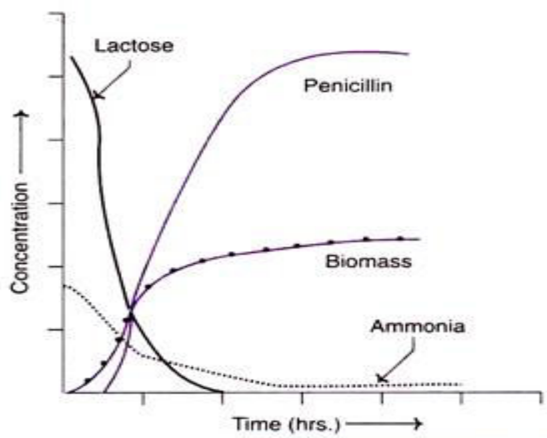

Fig. 25.4 : Penicillin production in relation to substrates utilization and biomass formation.

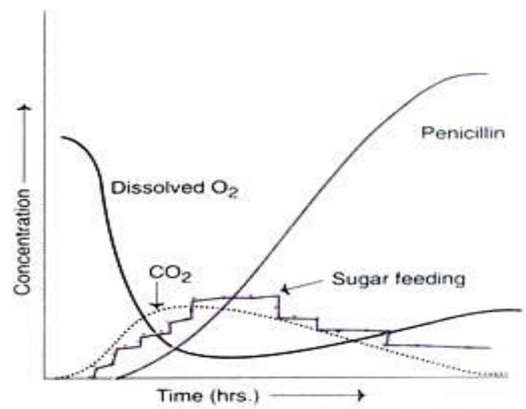

Fig. 25.5: Penicillin production in relation to continuous feeding of sugar, O_2 utilization, and CO_2 formation.

It is estimated that approximately 10% of the metabolised carbon contributes to penicillin production, while 65% is utilised towards energy supply and 25% for growth of the organisms. The efficiency of penicillin production can be optimized by adequate supply of carbon source. Thus, by adding glucose and acetic acid, the yield can be increased by about 25%.

For efficient synthesis of penicillin, the growth of the organism from spores must be in a loose form and not as pellets. The growth phase is around 40 hours with a doubling time of 6-8 hours. After the growth phase is stabilized, the penicillin production exponentially increases with appropriate culture conditions. The penicillin production phase can be extended to 150-180 hours.

Recovery of Penicillin:

As the fermentation is complete, the broth containing about 1% penicillin is processed for extraction. The mycelium is removed by filtration. Penicillin is recovered by solvent (n-butyl acetate or methyl ketone) extraction at low temperature (<10°C) and acidic pH (<3.0). By this way, the chemical and enzymatic (bacterial penicillinase) degradations of penicillin can be minimized.

The penicillin containing solvent is treated with activated carbon to remove impurities and pigments. Penicillin can be recovered by adding potassium or sodium acetate. The potassium or sodium salts of penicillin can be further processed (in dry solvents such as n-butanol or isopropanol) to remove impurities. The yield of penicillin is around 90%.

As the water is totally removed, penicillin salts can be crystallized and dried under required pressure. This can be then processed to finally produce the pharmaceutical dosage forms. Penicillins G and H are the fermented products obtained from the fungus Penicillium chrysogenum.

Production of 6-Amino Penicillanic Acid:

The penicillins G and H are mostly used as the starting materials for the production of several synthetic penicillins containing the basic nucleus namely 6-amino penicillanic acid (6-APA). About 10 years ago, only chemical methods were available for hydrolysis of penicillins to produce 6-APA. Nowadays, enzymatic methods are preferred.

Immobilized penicillin amidases enzymes have been developed for specific hydrolysis of penicillin G and penicillin V. Penicillin salt of either G or V can be used for hydrolysis by immobilized enzyme system. The pH during hydrolysis is kept around 7-8, and the product 6-APA can be recovered by bringing down the pH to 4.

At pH 4, 6-amino penicillanic acid gets precipitated almost completely in the presence of a water immiscible solvent. In general, the enzymatic hydrolysis is more efficient for penicillin V than for penicillin G. However, penicillin G is a more versatile compound, as it is required for ring expansions.