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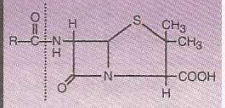
Microbial Production of Antibiotics



Antibiotics are the ***chemical substances that can kill microorganisms or inhibit their growth***, and are therefore used to fight infections in humans or animals. Most of the antibiotics are produced by microorganisms (i.e. product of one organism that can kill other organism). Certain semi-synthetic antibiotics are the chemically modified natural antibiotics.

Antibiotics have undoubtedly changed the world we live in, and have certainly contributed to the increase in the human life-span. This is mainly due to the fact that several life-threatening infectious diseases could be conveniently cured by administration of antibiotics.

ANTIBIOTICS — GENERAL

A brief history of antibiotics along with the microorganisms producing them, and their applications are given hereunder.

History of antibiotic discovery

It was in 1928, Alexander Fleming made an accidental discovery that the fungus *Penicillium notatum* produced a compound (penicillin) that selectively killed a wide range of bacteria without adversely affecting the host cells. There are records that in some parts of Europe (in 1908) extracts of moldy bread were applied to wounds or abrasions to prevent infections, although the biochemical basis was not known. The ***penicillin discovery of Fleming has revolutionised antibiotic research***.

Wide range of antibiotics

Antibiotics are the most important class of pharmaceuticals produced by microbial biotechnological processes. They are the ***products of secondary metabolism***.

Around 10,000 different antibiotics are known, and 200–300 new ones are being added each year. Most of these antibiotics are not of commercial importance due to various reasons—toxicity, ineffectiveness or high cost of production. There are around 50 antibiotics which are most widely used.

In ***Table 25.1***, a selected list of important antibiotics, their properties and the producing organisms is given.

Broad spectrum antibiotics : They can control the growth of several unrelated organisms. e.g. tetracyclines, chloramphenicol.

Narrow spectrum antibiotics : They are effective against selected species of bacteria e.g. penicillin, streptomycin.

Microorganisms producing antibiotics

A great majority of antibiotics are produced by ***actinomycetes*** particularly of the genus ***Streptomyces*** e.g. tetracyclines, actinomycin D.

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Table 25.1 A selected list of important antibiotics along with producing organisms

Antibiotic activity specturm and antibiotic	*Producing microorganism*
Antibacterial	
Penicillin G	*Penicillium* sp
Cephalosporin	*Acremonium* sp
Streptomycin	*Streptomyces* sp
Tetracycline	*Streptomyces* sp
Chloramphenicol	*Cephalosporium* sp
Bacitracin	*Bacillus* sp
Antitumor	
Actinomycin D	*Streptomyces* sp
Mitomycin C	*Streptomyces* sp
Bleomycin	*Streptomyces* sp
Adriamycin	*Streptomyces* sp
Daunomycin	*Streptomyces* sp
Antifungal	
Griseofulvin	*Penicillium* sp
Food preservative	
Natamycin	*Streptomyces* sp
Nisin	*Streptomyces* sp
Antiprotozoal	
Daunorubicin	*Steptomyces* sp
Antituberculosis	
Refamycin	*Nocardia* sp
Antiamoebic	
Tetracycline	*Streptomyces* sp
Fumagillin	*Aspergillus* sp

The bacteria other than actinomyces also produce certain antibiotics e.g. bacitracin.

Among the ***fungi***, the two groups ***Aspergillaceae*** and ***Moniliales*** are important for antibiotic production e.g. penicillin, cephalosporin, griseofulvin.

APPLICATIONS OF ANTIBIOTICS

Antibiotics are particularly important ***as antimicrobial agents for chemotherapy***. A large number of bacterial diseases have been brought under control by use of antibiotics. These include pneumonia, cholera, tuberculosis and leprosy. The antifungal antibiotic griseofulvin has controlled the debilitating fungal skin diseases such as ring worm. Besides serving as antimicrobial agents, there are several other applications of antibiotics.

Antitumor antibiotics : There are a selected few antibiotics that are in use for control of cancer growth, although with a limited success e.g. actinomycin D, mitomycin C.

Food preservative antibiotics : Certain antibiotics are used in canning industry (e.g. chlortetracycline), and for preservation of fish, meat and poultry (e.g. pimaricin, nisin). The use of antibiotics in food preservation is usually under the control of the Governments.

Antibiotics used in animal feed and veterinary medicine : Till some time ago, antibiotics (penicillins, tetracyclines, erythromycins) were very widely used in processing of animal feeds. Such an indiscriminate use resulted in the development of antibiotic resistance in animals and humans. A new class of antibiotics have been developed for specific use in animal feed e.g. enduracidin, tylosin. Likewise, specific antibiotics have been developed for exclusive use in veterinary medicine e.g. hygromycin B, theostrepton, salinomycin.

Antibiotics for control of plant diseases : In recent years, several antibiotics have been developed for exclusive use to control plant diseases e.g. blasticidin, tetranactin, polyoxin.

Antibiotics as tools in molecular biology : Some of the antibiotics can selectively inhibit certain biological reactions at the molecular level. These antibiotics do in fact serve as tools for exploring the knowledge of life sciences. Thus, certain antibiotics have been used to obtain some important information on DNA replication, transcription and translation.

PRODUCTION OF ANTIBIOTICS — A MAJOR PHARMACEUTICAL INDUSTRY

The commercial production of antibiotics is a highly profitable industry worldover. Annual sales of antibiotics will run into several billions of dollars with an annual growth potential of about 10%.

Antibiotics may be ***produced by microbial fermentation***, or ***chemical synthesis***, or a ***combination of both***. For certain antibiotics, the

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basic molecule is produced by fermentation and its therapeutic value can be increased by chemical modifications. The cost involved in production and chemical modifications, besides the efficacy of the antibiotic is very important in its manufacture.

Biotechnologists continue their efforts to increase the fermentation yield and recovery processes to produce pure antibiotics.

The industrial production of selected antibiotics is briefly described in the following pages.

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.

Action of penicillins

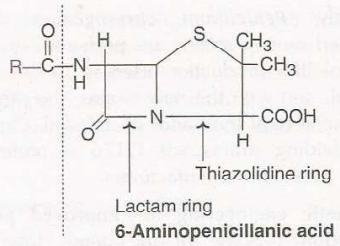
Natural penicillins (penicillins V and G) are ***effective against several Gram-positive bacteria***. They inhibit the bacterial cell wall (i.e. peptoglycan) synthesis and cause cell death. Some persons (approximately 0.5-2% of population) are allergic to penicillin.

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 cephalosporins.

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, ***Penicillium notatum*** was used for the large-scale production of penicillins.



6-Aminopenicillanic acid

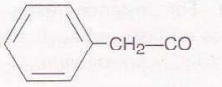
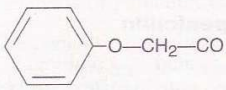
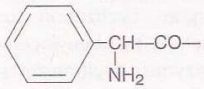
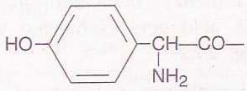
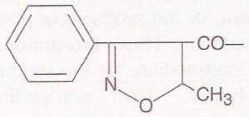
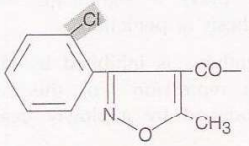
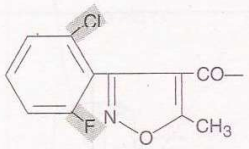
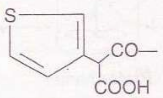
R-group	Name of the penicillin
Biosynthetic penicillins	
	Penicillin G (benzylpenicillin)
	Penicillin V
Semi-synthetic penicillins	
	Ampicillin
	Amoxicillin
	Oxacillin
	Cloxacillin
	Floxacillin
	Ticarcillin

***Fig. 25.1** : Structures of important penicillins.*

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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 phenylacetyl 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***).

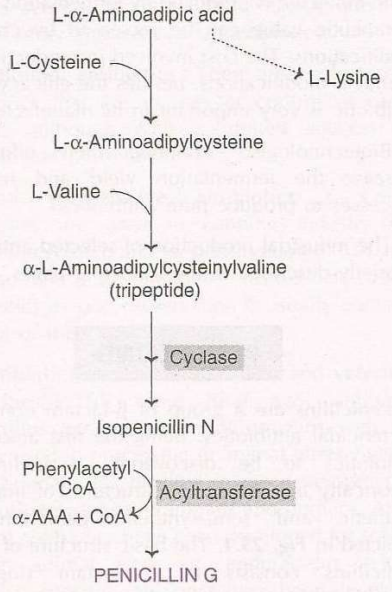

Fig. 25.2 : *Biosynthesis of penicillin by* Penicillium chrysogenum *(α-AAA — α-Amino adipic acid; CoA–Coenzyme A.*

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 prefermenter, and then to fermenter.

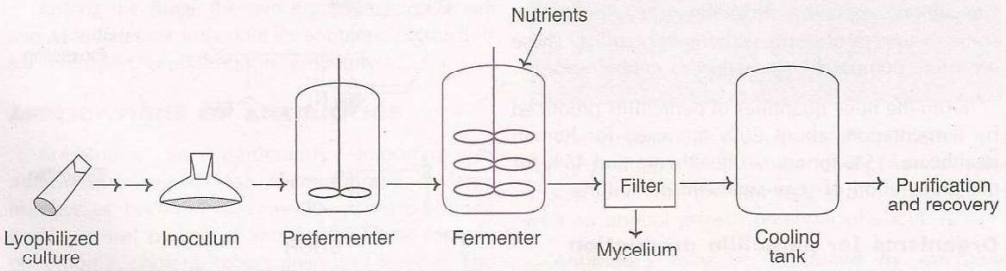

Fig. 25.3 : *An outline of the flow chart for penicillin fermentation.*

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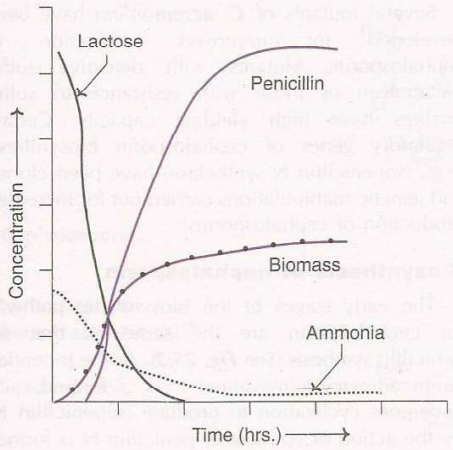

Fig. 25.4 : Penicillin production in relation to substrates utilization and biomass formation.

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. Pencillin 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***.

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-butylacetate or methylketone) 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.

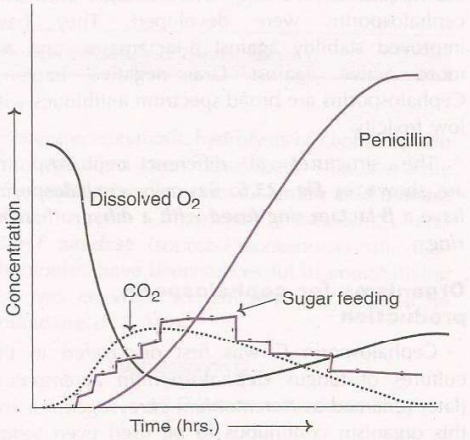

Fig. 25.5 : Penicillin production in relation to continuous feeding of sugar, O_2 utilization, and CO_2 formation.

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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. Now a days, 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.

CEPHALOSPORINS

The pharmaceutical uses of penicillins are associated with allergic reactions in some individuals. To overcome these allergic problems, cephalosporins were developed. They have improved stability against β-lactamases, and are more active against Gram-negative bacteria. Cephalosporins are broad spectrum antibiotics with low toxicity.

The structures of different cephalosporins are shown in ***Fig. 25.6***. Basically ***cephalosporins have a β-lactam ring fused with a dihydrothiazine ring***.

Organisms for cephalosporin production

Cephalosporin C was first discovered in the cultures of fungus *Cephalosporium acremonium* (later renamed as *Acremonium chreysogenum*) and this organism continuous to be used even today. The other organisms employed for cephalosporin production are *Emericellopsis* sp, *Paecilomyces* sp and *Streptomyces* sp.

Several mutants of ***C. acremonium*** have been developed for improved production of cephalosporin. Mutants with defective sulfur metabolism or those with resistance to sulfur analogs have high yielding capacity. Certain regulatory genes of cephalosporin biosynthesis (e.g., isopenicillin N synthetase) have been cloned and genetic manipulations carried out for increased production of cephalosporins.

Biosynthesis of cephalosporin

The early stages of the biosynthetic pathway for cephalosporin are the same as that for penicillin synthesis (See ***Fig. 25.2***). As the tripeptide (aminoadipylcysteinylvaline) is formed, it undergoes cyclization to produce isopenicillin N. By the action of epimerase, penicillin N is formed from isopenicillin N. Then, penicillin N gets converted to cephalosporin C by a three stage reaction catalysed by three distinct enzymes namely expandase, hydroxylase and acetyl transferase (***Fig. 25.7***).

Regulation of biosynthesis : A low concentration of lysine promotes cephalosporin synthesis. The inhibitory effect of lysine at a higher concentration can be overcome by adding L-aminoadipic acid. The carbon sources that get rapidly degraded (e.g. glucose, glycerol) reduce cephalosporin production. Methionine promotes cephalosporin synthesis in *C. acremonium*, but has no influence on *Streptomycetes*.

PRODUCTION PROCESS OF CEPHALOSPORIN

The fermentation process concerned with the production of cephalosporin is similar to that of penicillin. The culture media consists of corn steep liquor and soy flour-based media in a continuous feeding system. The other ingradients of the medium include sucrose, glucose and ammonium salts. Methionine is added as a source of sulfur.

The fermentation is carried out at temperature 25–28°C and pH 6-7. The growth of micro-organisms substantially increases with good O_2 supply, although during production phase, O_2 consumption declines.

Cephalosporin C from the culture broth can be recovered by ion-exchange resins, and by using column chromatography. Cephalosporin C can be precipitated as zinc, sodium or potassium salt, and isolated.

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