ANTIBIOTICS

Greek word *Anti* – against *Bios* – life

According to K.D Tripathi- 5TH Edition, Antibiotics are substances produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations.

According to Waksman, Antibiotics are chemical substances produced by microorganisms having the property of inhibiting the growth or destroying other microorganisms in high dilution.

In general, antibiotics that are commercially useful can be divided into a few major classes. This is normally based on their biosynthetic routes and precursors used by the organism to make these compounds, often followed by specific and unique biosynthetic modifications. The main classes include:

- antibiotics derived from amino acid precursors either made through a non-ribosome synthetic pathway (beta-lactams, cyclic peptides, lipopetides) or those based on a normal ribosome-based synthetic route (nisins);
- antibiotics manufactured from sugars (aminoglycosides);

Classification

depending on chemical structure

1. β-lactum antibiotics (Penicillin, Cephalosporin)

2. Nitrobenzene derivative (Chloramphenicol)

3. Aminoglycosides (Streptomycin)

Type of organisms against which primarily active

a) Antibacterial- Penicillin, Aminoglycosides

b) Antifungal- Griseofulvin, Amphotericin B etc

c) Antiviral- Acyclovir, Zidovudine

Spectrum of Activity

a) Narrow Spectrum – Penicillin–G, Streptomycin

b) Broad Spectrum - Chloramphenicol.

Spectrum Antibiotic

Narrowest antibacterial spectrum should be chosen.

1. It minimizes the risk of superinfection.

2. Broad-spectrum antibiotic develop resistant against many bacteria.

3. Use of narrow-spectrum antibiotics allow larger proportion of the host flora to be maintained, by reducing superinfection to minimum.

Source of Antibiotics

a) Fungi- Penicillin

b) Bacteria- polymyxin B, Bacitracin

c) Actinomycetes- Aminoglycosides.

Antibiotics are low-molecular-weight organic compounds produced by microorganisms that at low concentrations are able to selectively inhibit the growth of other microorganisms. These compounds are considered secondary metabolites, since they are not required for normal growth, development, or reproduction of the microorganisms that produce them. The biological role of antibiotics is controversial and it has been suggested that antibiotic production may confer an ecological advantage for survival in natural habitats where nutrients are limiting for microbial growth.

The discovery of β-lactam antibiotics is one of the most significant milestones in human history and it entailed a revolution in modern chemotherapy. The members of the family of β-lactam antibiotics stand out from the other family members because of their high activity and low toxicity, for which they are among the most commonly prescribed drugs. The use of these compounds has helped medicine to face up to infectious diseases, which have been the cause of death of millions of human beings throughout history, and dramatically reduce the mortality rate.

Overview of β-lactam antibiotics

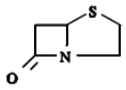
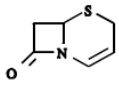
The history of β-lactam antibiotics is one marked by serendipity and chance. Alexander Fleming noticed in a Petri-dish antibiosis between a contaminant mould, P. notatum. Fleming cultured the mould and showed that broth filtrates had antimicrobial activity against several species of Gram-positive bacteria. In the UK penicillin was initially isolated from surface cultures of P. notatum strains, submerged fermentation was applied for the first time in 1941 by the Northern Regional Research Laboratory (NRRL, Preoria, Illinois). After a worldwide search for high-producing natural isolates, the parental P. chrysogenum strain NRRL 1951 was selected as a source of more productive sub-strains.

There are two major classes of microbially produced beta-lactam antibiotics that are still manufactured in quantity, the penicillins and cephalosporins. β-Lactam antibiotics (penicillin, ampicillin, cephalosporins, etc.) are narrow- and moderate-spectrum antibiotics because some members are only effective against Gram-positive organisms while other members can also kill certain Gram-negative bacteria.

Penicillins and cephalosporins belong to the large and complex family of β- lactam antibiotics which members possess one common structural motif, the four membered β-lactam ring. The β -lactam ring enables these antibiotics to selectively inhibit enzymes involved in the cell-wall assembly of primarily Gram-positive bacteria. In penicillins, the β -lactam ring is fused with a five-membered thiazolidine ring. In cephems, i.e. cephalosporin C, cephamycins and cephabacins, the second cyclic structure is the six-membered dihydrothiazine ring. While hydrophobic penicillins are solely produced by filamentous fungi, cephems are produced by filamentous fungi and bacteria. Hydrophobic penicillins and cephalosporin C are regarded classical fungal secondary metabolites.

Penicillins and the structurally related cephalosporin C are natural products of filamentous fungi. From these, *Penicillium chrysogenum* and *Acremonium chrysogenum* are used for the biotechnological bulk production of penicillins and cephalosporin C, respectively. Only the penicillins G and V are produced in industry by fed-batch cultivation of high-yielding *P. chrysogenum* strains.

Table 1.1 Distribution of β -lactam producing microorganisms in nature

Class of β-lactam	Ring structure	Fungi	Bacteria	
			Gram-Positive	Gram-negative
Penam		*Aspergillus* *Penicillium* *Epidermophyton* *Trichophyton* *Polypaecilum* *Malbranchea* *Sartorya* *Pleurophomopsis*		
Cephem		*Acremonium* *Anixiopsis* *Arachnomyces* *Spiroidium* *Scopulariopsis* *Diheterospora* *Paecilomyces*	*Streptomyces* *Nocardia*	*Flavobacterium* *Xanthomonas* *Lysobacter*

Structure and Mechanism of Action of Penicillins and Cephalosporins

The β-lactam antibiotics, like many other secondary metabolites, have unusual chemical structures. All β-lactams contain a four-membered β-lactam ring closed by an amide bond.

The first microbially produced antibiotics to be discovered were the penicillins. Penicillins contain a bicyclic 'penam' nucleus (Figure 1) formed by fused β-lactam and sulfur-containing thiazolidine rings and an acyl side chain, which depends on the precursors present in the culture medium, bound to the amino group at C-6. Hydrophobic penicillins are exclusively synthesized by filamentous fungi from the genera Penicillium (e.g., P. chrysogenum) and Aspergillus (Aspergillus nidulans), whereas hydrophilic penicillins are synthesized by filamentous fungi (A. chrysogenum), actinomycetes (Streptomyces sp.), and some Gram-negative bacteria.

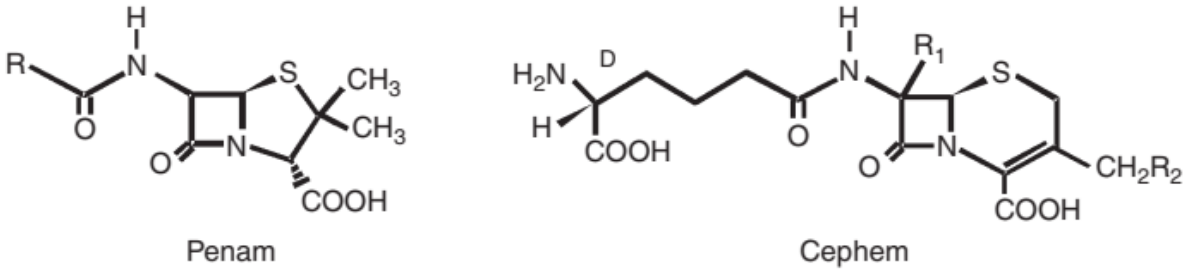
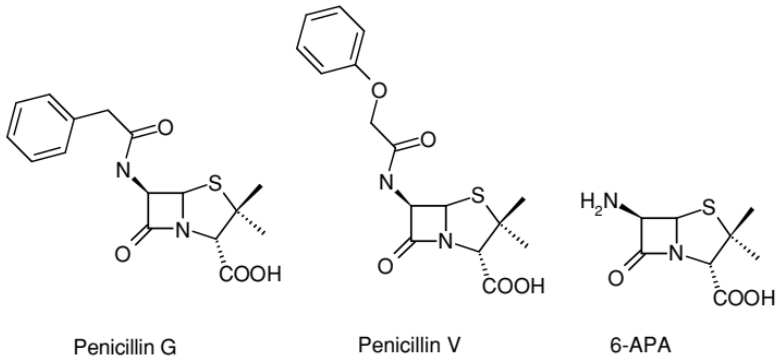


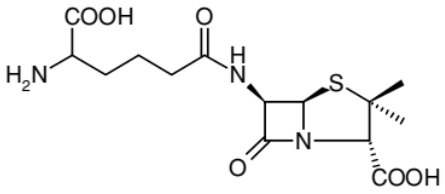
Figure 1 Chemical structure of the penicillins (penam nucleus) and cephalosporins (cephem nucleus). The D-configuration of the α-aminoadipic side chain is indicated on carbon 1 of this amino acid by D.

Cephalosporins contain the 'cephem' nucleus (Figure 1), a six-membered dihydrothiazine ring fused to the β-lactam ring. Cephalosporin C has a D-α-aminoadipyl side chain attached to the C-7 amino group, which is identical to that of hydrophilic penicillin N but differs from that of hydrophobic penicillins. Cephalosporins are produced by the fungi A. chrysogenum, Paecilomyces persicinus, Kallichroma tethys, and some other deuteromycetes, although Gram-positive actinomycetes such as Streptomyces clavuligerus or Nocardia lactamdurans and Gram-negative bacteria such as Lysobacter lactamgenus also synthesize cephalosporins as intermediates of cephamycin and cephabacin biosynthetic pathways.

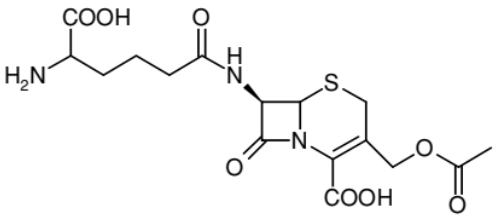


Penicillin G and V share a common bicyclic ring structure, with the β-lactam portion of the molecule being responsible for the biological activity. New penicillins are made by taking this basic nucleus in the form of 6-aminopenicillanic acid (6-APA).

Cephalosporin C is more stable than penicillin N and this allowed industrial strain-improvement programmes to develop strains of *Cephalosporium* that produce very high titres of cephalosporin C.



Penicillin N



Cephalosporin C

The cephalosporins are classified into generations based on their general features of antimicrobial activity. First-generation cephalosporins include agents with good activity against Gram-positive bacteria (*S. aureus*, group A streptococci, *Streptococcus pneumoniae*) and relatively modest coverage for Gram-negative organisms. Second generation antimicrobials have increased activity against certain Gram-negative pathogens, including *Haemophilus influenzae, Neisseria meningitidis* and *Moraxella catarrhalis*. Third-generation cephalosporins are somewhat less active against Gram-positive cocci, but much more active against enteric Gram-negative organisms.

Penicillin and Cephalosporin Biosynthetic Pathways

The biosynthesis of β-lactam compounds (Figure 2) involves sequential reactions including the formation of the penam nucleus from a linear tripeptide that is cyclized (early biosynthetic steps), ring expansion of the penam to the cephem nucleus (intermediate steps), and modification of the β-lactam nucleus (late decorating steps).

The basic structure of classical β-lactam antibiotics originates from three amino acids: L-α-aminoadipic acid, L-cysteine, and L-valine. L-Valine and L-cysteine are common amino acids, unlike L-α-aminoadipic acid, which is a nonproteinogenic amino acid formed by a specific pathway related to the fungal lysine biosynthetic pathway. The two early enzymatic steps are common to all classical β-lactam producers. These steps lead to the biosynthesis of isopenicillin N (IPN), which is the first compound in the pathway with antibiotic activity. The first enzyme of the pathway is the nonribosomal peptide synthetase L-δ(α-aminoadipyl)-L-cysteinyl-D-valine (ACV) synthetase (ACVS), which is a very large multifunctional protein (MW around 420 kDa). This protein is encoded by a single structural 11 kb intronless gene called pcbAB (acvA), which is present in both fungal and bacterial penicillin and cephalosporin (and cephamycin) gene clusters. The ACVS consists of three well-conserved domains that activate each of the three amino acids. This protein sequentially activates the three substrate amino acids with ATP as aminoacyl adenylates, binds them to the enzyme as thioesters, epimerizes the L-valine to D-valine, catalyzes the condensation of the three amino acids to form the tripeptide ACV, and, finally, releases this tripeptide from the enzyme by means of the internal thioesterase activity. In the second step of the early biosynthetic stage, the IPN synthase (cyclase) encoded by the intronless pcbC (ipnA) gene, which is an intermolecular dioxygenase that requires Fe^{2+}, molecular oxygen, and ascorbate, catalyzes the removal of four hydrogen atoms from the ACV tripeptide. This reaction leads to the oxidative ring

closure of the tripeptide and the formation of the bicyclic structure (penam nucleus) of IPN, which constitutes the branch point of penicillin and cephalosporin biosynthesis.

Hydrophobic penicillin-producing fungi (e.g., P. chrysogenum and A. nidulans) contain, in addition to the pcbAB (acvA) and pcbC (ipnA) genes common to filamentous fungi and bacteria, a third gene in the penicillin cluster. This gene, named penDE (aatA), encodes the peroxisomal acyl CoA: IPN acyltransferase (IAT) and it is not present in cephalosporin C- or cephamycin-producing microorganisms. One distinctive feature of the penDE (aatA) gene is that unlike the other two genes in the cluster, it contains three introns, which suggests a eukaryotic origin. The IAT is synthesized as a 40-kDa precursor protein (proIAT) that undergoes anautocatalytic processing between residues Gly102 and Cys103. The active protein is a heterodimer of two subunits: α (11 kDa, corresponding to the N-terminal fragment) and β (29 kDa, corresponding to the C-terminal region). This heterodimeric enzyme removes the α-aminoadipic side chain of IPN and exchanges it for hydrophobic acyl molecules, which have to be previously activated by aryl-CoA ligases before they become substrates for the IAT. Due to the broad specificity of IAT and to the presence of several isoenzymes, a wide range of side chains may serve as substrates for this enzyme. Thus, natural penicillins, such as penicillin F (D3-hexenoic as side chain) and K (octanoic acid as side chain), are synthesized under natural conditions. However, feeding the cultivation media with phenylacetic or phenoxyacetic acids directs the biosynthesis mainly toward benzylpenicillin (penicillin G) or phenoxymethylpenicillin (penicillin V), respectively. In addition to the aatA gene, A. nidulans possesses another gene, which has been recently characterized. This gene, named aatB, is not clustered with the rest of the penicillin genes and encodes cytosolic protein, likely an acyl-CoA transferase, which plays a role in penicillin biosynthesis. The P. chrysogenum aatB homologue (named ial), however, appears to differ in function from that of A. nidulans, since the activity of the protein encoded by this gene was not related to penicillin biosynthesis.

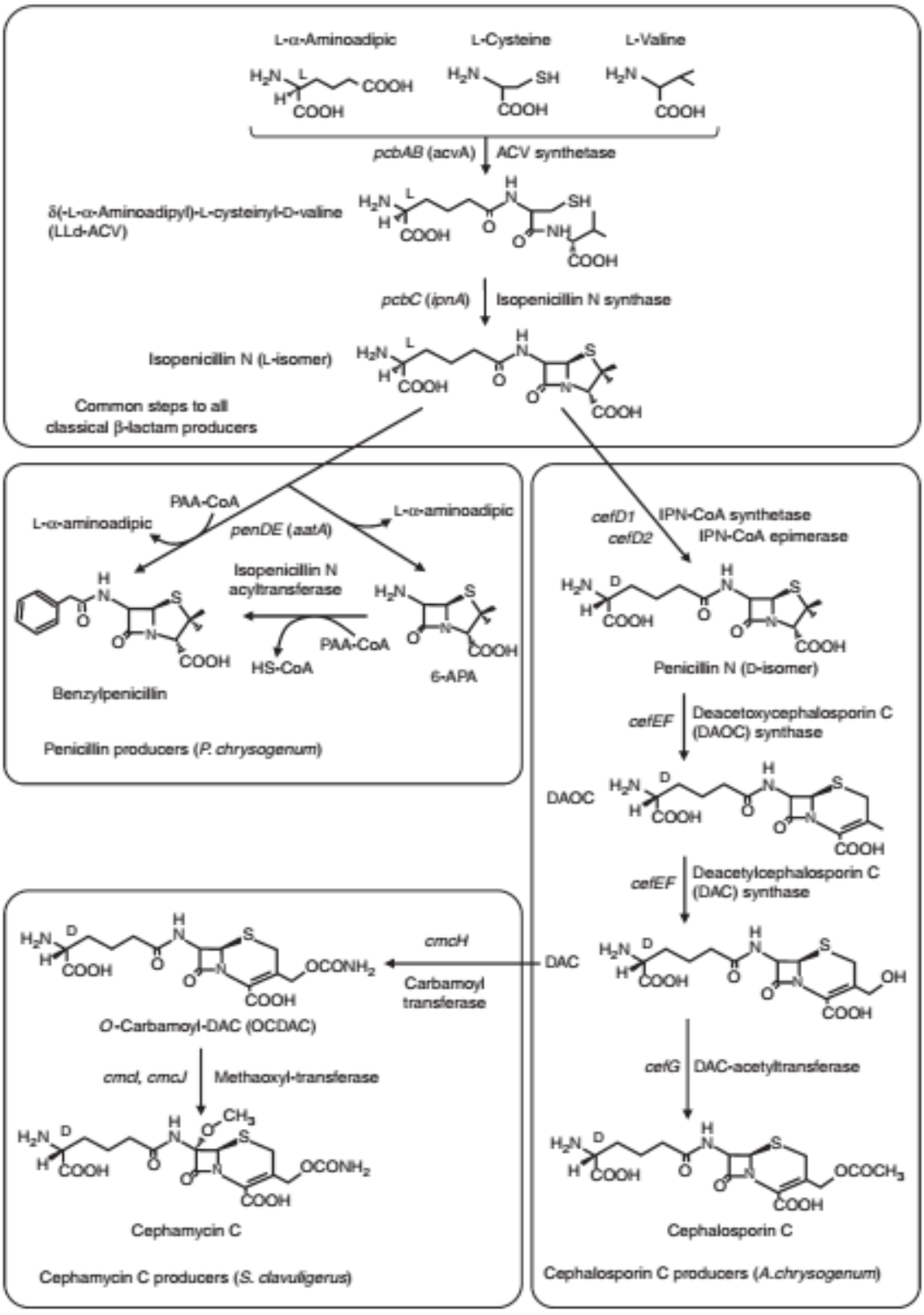


Figure 2 Biosynthetic pathways of benzylpenicillin, cephalosporin C, and cephamycin C in P. chrysogenum, A. chrysogenum, and S. clavuligerus, respectively. The first two steps (upper part of the figure) are common to all classical β-lactam producers. The L- or D-configuration of the α-aminoadipic side chain is indicated on carbon 1 of this amino acid by L or D.

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[The information, including the figures, are collected from the above references and will be used solely for academic purpose.]