Microbially production of Antibiotics Penicillin

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 antibiotics are produced by microorganisms (i.e. products of one organism that can kill other organisms). 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 antibiotic.

History of antibiotic discovery

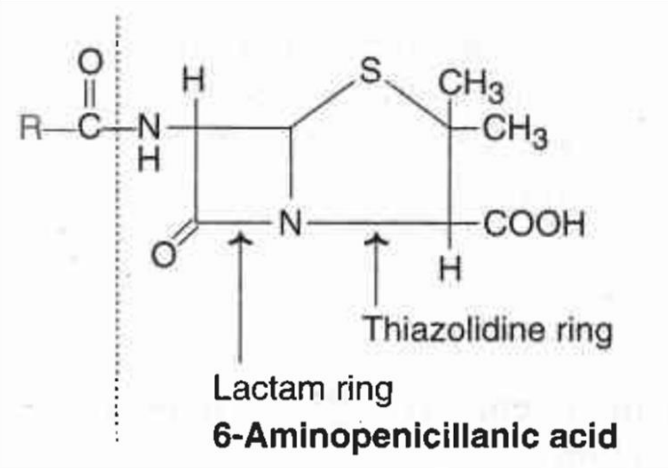
It was in 1928, **Alexander Fleming** made an accidental discovery that the fungus ***Penicillium notatum*** produced a compound (penicillin) that selectively killed a wide range of bacteria without adversely affecting the host cells.

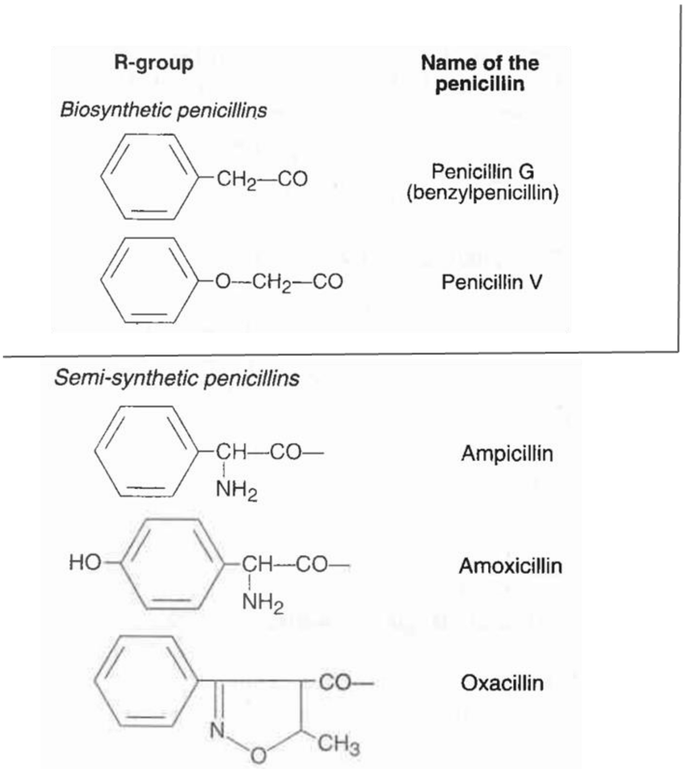
There are records that in some parts of Europe (in 1908) extracts of moldy bread were applied to wounds or abrasions to prevent infections, although the biochemical basis was not known. The penicillin discovery of Fleming has revolutionized antibiotic research.

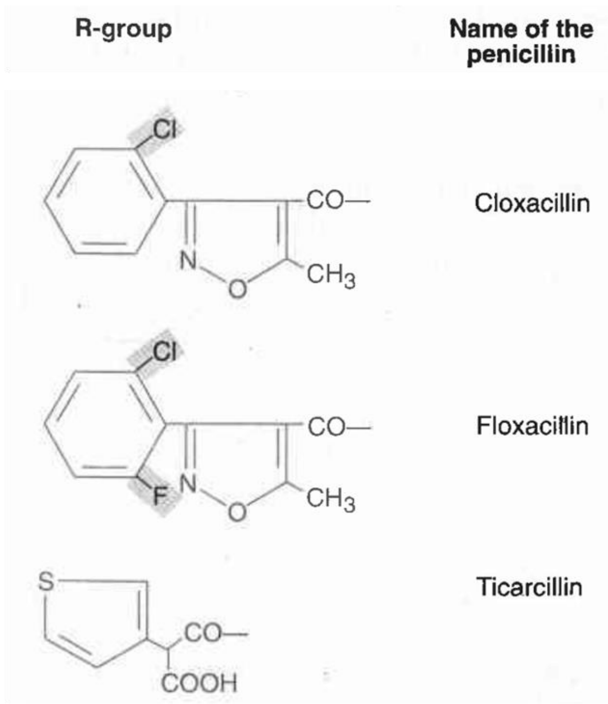
Penicillin

Penicillins are a group of **β-lactam** containing bactericidal antibiotics.

The basic structure of all the penicillins consists of a **lactam ring** and a **thiazolidine ring** fused together to form **6-aminopenicillanic acid**.







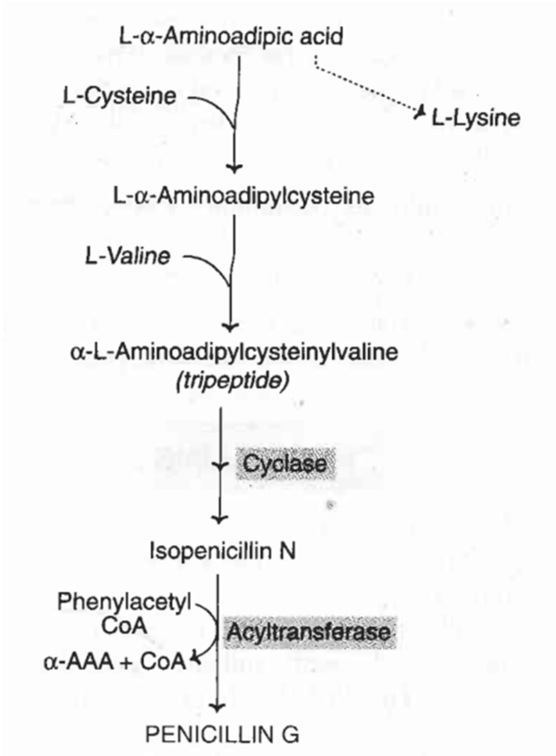
Organisms for penicillin production

- In the early days, ***Penicillium 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 manufacturer.

  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-viline** 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.



Regulation of biosynthesis :

- 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 degrade sugar like **lactose.**
- The concentrations of phosphate and ammonia also influence penicillin synthesis.

PRODUCTION PROCESS OF PENICILLIN

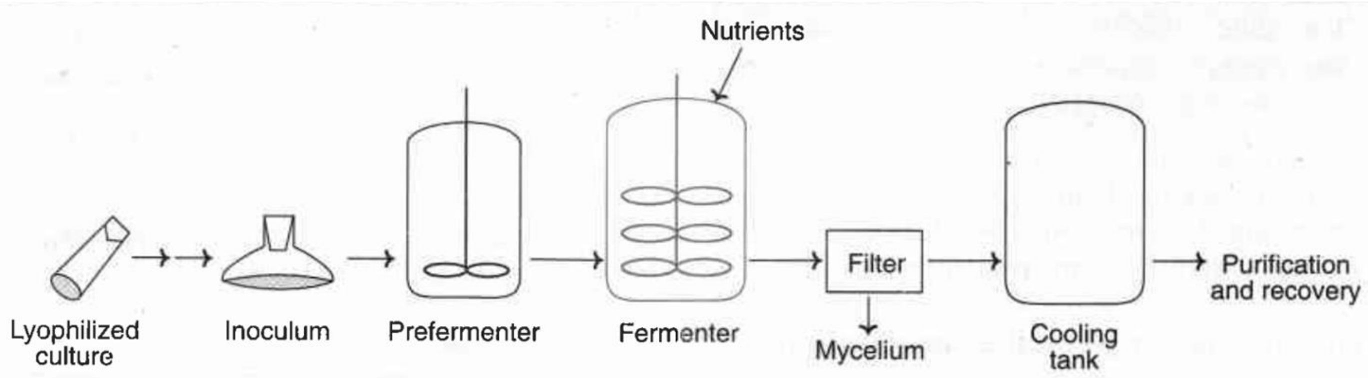


Fig: An outline of the flow chart for the industrial production of penicillin

The lyophilized culture of spores is cultivated for inoculum development which is transferred to prefermenter, and then to fermenter.

Culture Media

- 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.

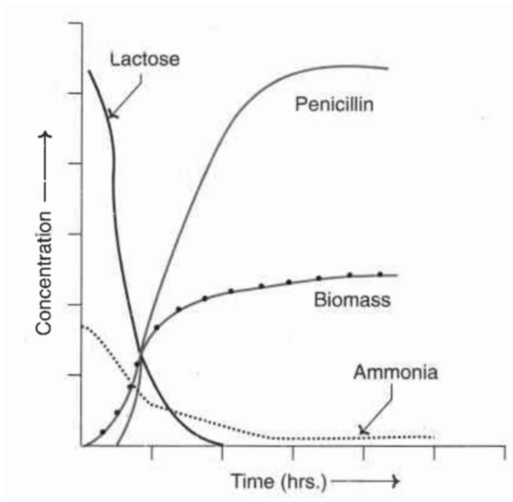


Fig: Penicillin production in relation to substrates utilization and biomass formation.

- 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. 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 completed, 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 ($<10^0$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***.

Applications of penicillins

- Natural penicillins (penicillins V and G) are effective against several Gram-positive bacteria. They inhibit the bacterial cell wall (i.e. peptidoglycan) 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.
- 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 penicillin.