Penicillin Production

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INTRODUCTION

Penicillin is a group of antibiotics that are commonly used to treat different types of gram positive and gram negative bacterial infections. In their structure, **beta-lactam** ring is located due to this reason these drugs are also called as **beta-lactam antibiotics**.

- Penicillin is derived from the Penicillium mould.
- It destroys bacteria by inhibiting the enzymes responsible for the formation of the cell wall in the bacterial cells.

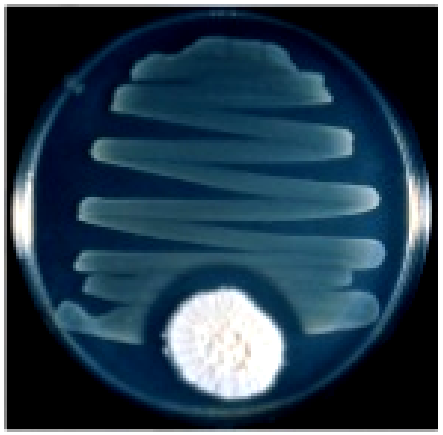
Penicillin Mold Fungus

History

- 1928: Scottish biologist, Alexander Fleming discovered that the *Staphylococcus* culture he had mistakenly left growing in open was contaminated with a mould which had destroyed the bacteria.

A. Fleming

- After isolating a sample and testing it, he found that it belonged to the *Penicillium* family.
  Later the mould was classified as *Penicillium notanum*.



- At first, it was difficult to convince people about its potential uses.

...History

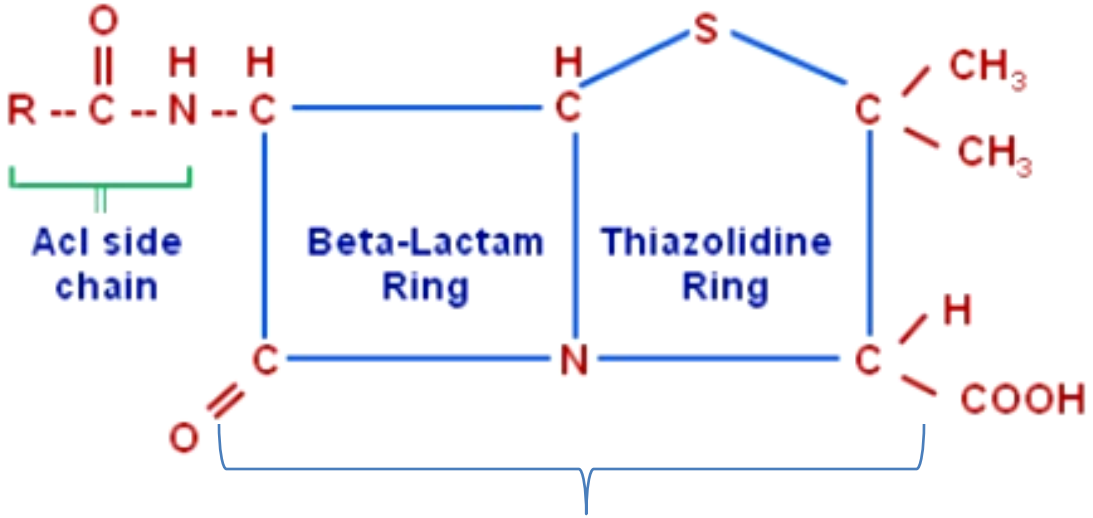
- But later (1939), using Fleming's work, two medical researchers, Howard Florey and Ernst Chain managed to purify penicillin in a powdered form.
- 1941: They successfully treated a human.
- 1943: They produced penicillin on a large scale. This helped immensely to treat casualties during the WWII that had bacterial infections due to their wounds.

Fleming, Florey and Chain received a Nobel prize in **1945** for medicine for their work on penicillin.

General structure of penicillin

- Have β-Lactam functional group, thus belong to the β-Lactam antibiotic group.

- They all have a basic ring-like structure (a β-Lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group (R).
  The nature of this acyl group produces specific properties on different types of penicillin.



6-Aminopenicillanic acid (6-APA)

Mechanism of action

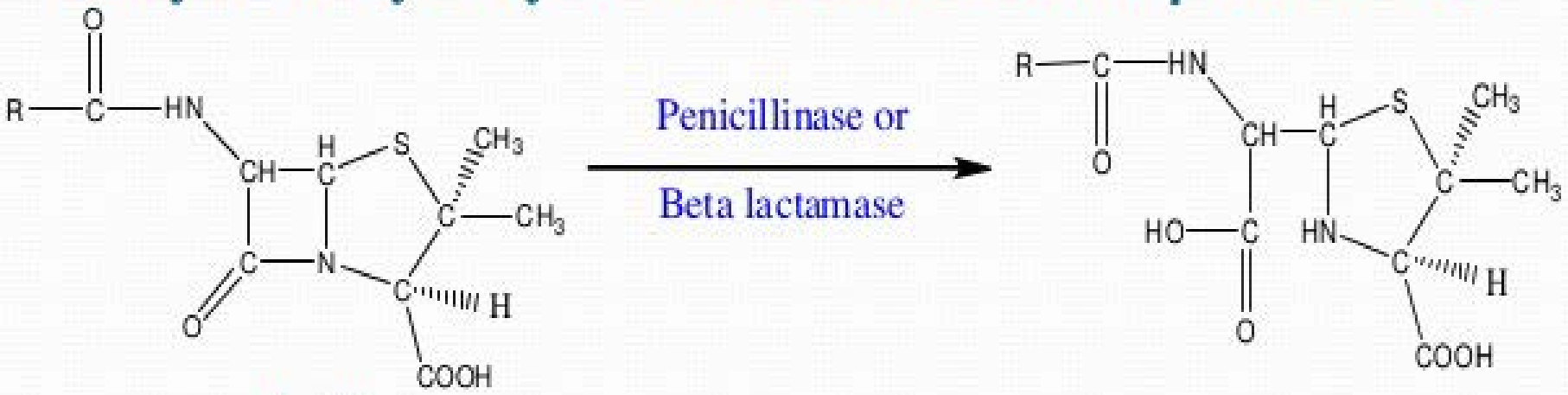
- Inhibits the synthesis of peptidoglycan in cell walls.
  - β-Lactam of penicillin binds to the enzyme - transpeptidase, that is used in the formation of peptidoglycan cross linking.
  - The enzyme is inhibited, thus inability to form cross linking.
  - Cell wall is weakened causing osmotic imbalance in the cell. This leads to cell death.

- As human cells do not have cell walls, penicillin does not affect them.

Penicillins sensitivity to microbial enzymes

- Penicillins are attacked by two enzymes of microbic origin.
- One is acylase or amidase, which simply severs the side-chain leaving the nucleus intact, a reaction which is now the first step in producing the many new semisynthetic penicillins.
- The second is penicillinase, which opens the β-lactam ring of penicillin and deprives it of all antibacterial activity.
- It was recognized more than 20 years ago that this enzyme was formed by some strains of staphylococci, and that they owed to this their resistance to the antibiotic.
- These strains have little " intrinsic " resistance ; it is not that they can tolerate penicillin, but that they can destroy it.
- Methicillin, Cloxacillin, Dicloxaciin, Oxacillin, Flucloxacillin, Nafcillin (penicillins) are resistant to penicillinase. Their side chain protect β-lactam ring from penicillinase.

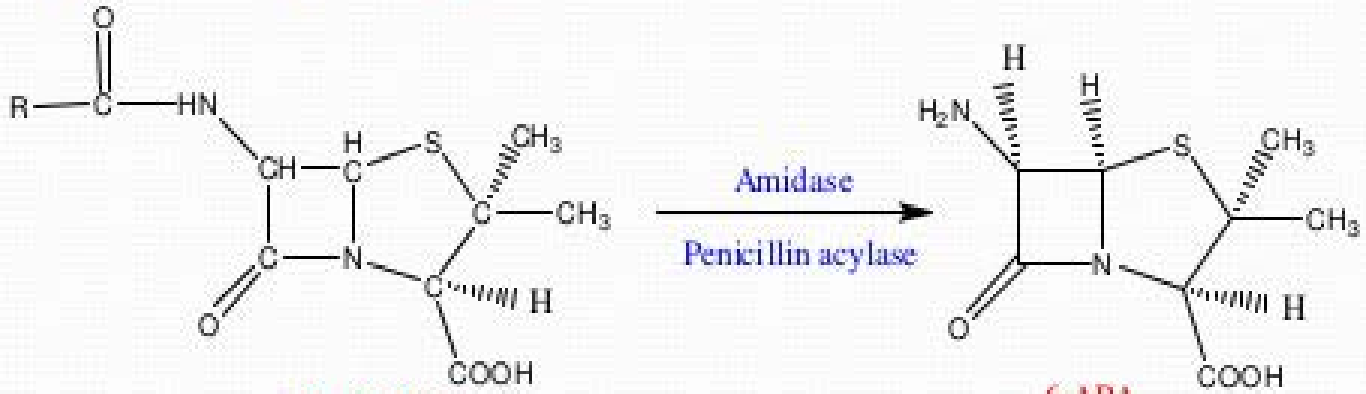
Enzymatic hydrolysis with Penicillinase or β lactamase:



Penicillin

Penicilloic acid

Enzymatic hydrolysis with Amidase:



Penicillin

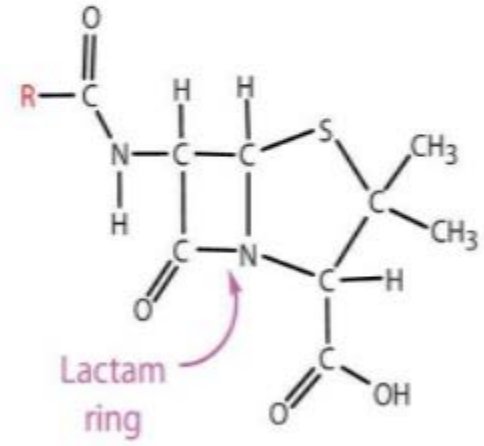
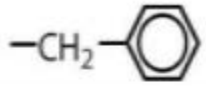
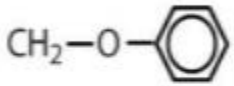
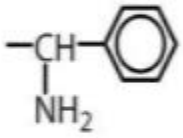
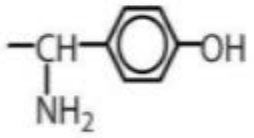
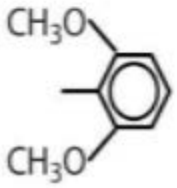
6-APA

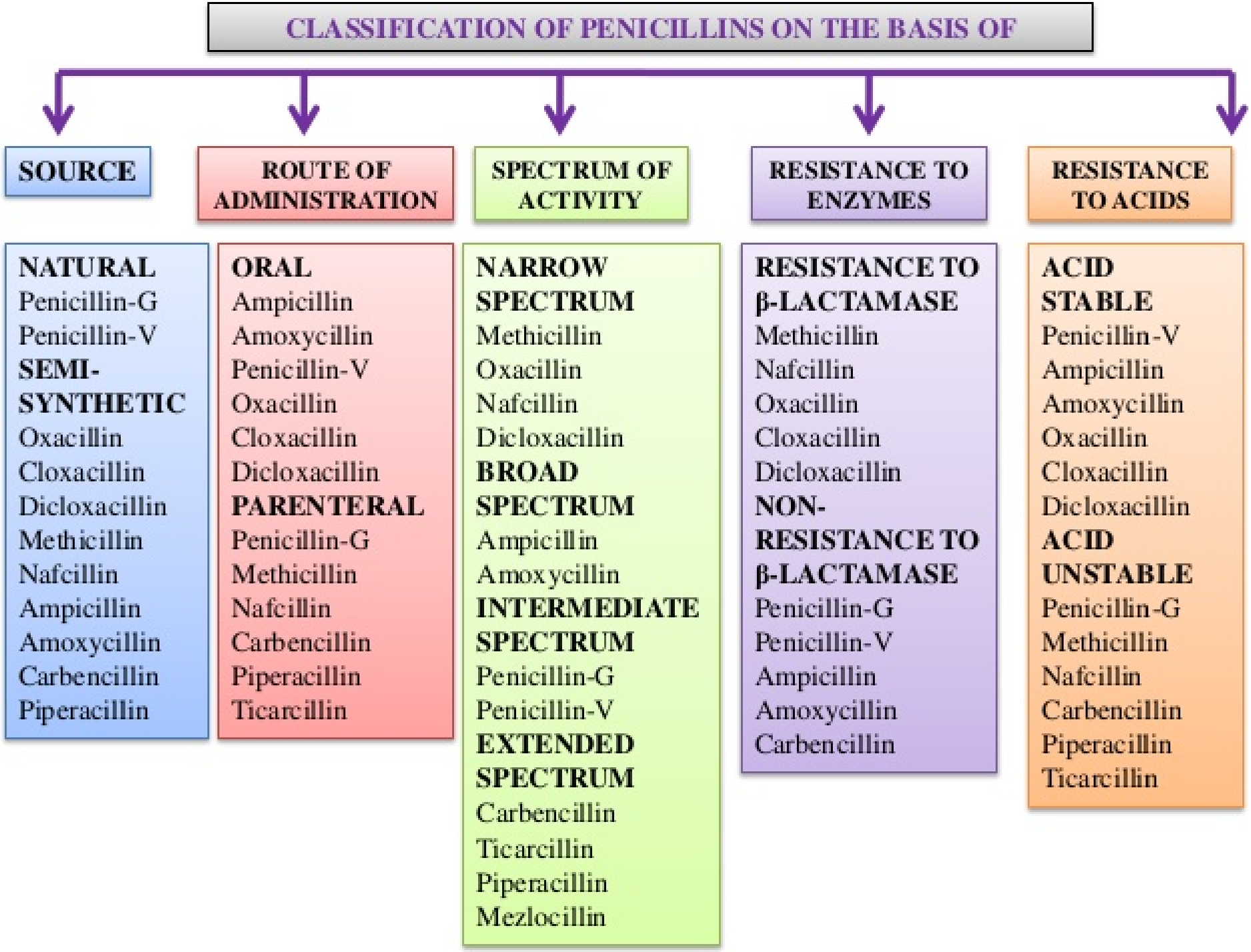
+

RCOOH

Penicillin derivatives

- Derivatives are produced to deal with the problem of bacterial resistance to penicillin.
- All penicillins have a constant core region which is the 6-APA.
- The only region that is different among penicillins is its R group.

Penicillin Structure	R Group	Drug Name
		penicillin G
		penicillin V
		ampicillin
		amoxicillin
		methicillin


CLASSIFICATION OF PENICILLINS ON THE BASIS OF
SOURCE
NATURAL
Penicillin-G
Penicillin-V
SEMI-SYNTHETIC
Oxacillin
Cloxacillin
Dicloxacillin
Methicillin
Nafcillin
Ampicillin
Amoxycillin
Carbencillin
Piperacillin
ROUTE OF ADMINISTRATION
ORAL
Ampicillin
Amoxycillin
Penicillin-V
Oxacillin
Cloxacillin
Dicloxacillin
PARENTERAL
Penicillin-G
Methicillin
Nafcillin
Carbencillin
Piperacillin
Ticarcillin
SPECTRUM OF ACTIVITY
NARROW SPECTRUM
Methicillin
Oxacillin
Nafcillin
Dicloxacillin
BROAD SPECTRUM
Ampicillin
Amoxycillin
INTERMEDIATE SPECTRUM
Penicillin-G
Penicillin-V
EXTENDED SPECTRUM
Carbencillin
Ticarcillin
Piperacillin
Mezlocillin
RESISTANCE TO ENZYMES
RESISTANCE TO β-LACTAMASE
Methicillin
Nafcillin
Oxacillin
Cloxacillin
Dicloxacillin
NON-RESISTANCE TO β-LACTAMASE
Penicillin-G
Penicillin-V
Ampicillin
Amoxycillin
Carbencillin
RESISTANCE TO ACIDS
ACID STABLE
Penicillin-V
Ampicillin
Amoxycillin
Oxacillin
Cloxacillin
Dicloxacillin
ACID UNSTABLE
Penicillin-G
Methicillin
Nafcillin
Carbencillin
Piperacillin
Ticarcillin

Strains used in penicillin fermentation

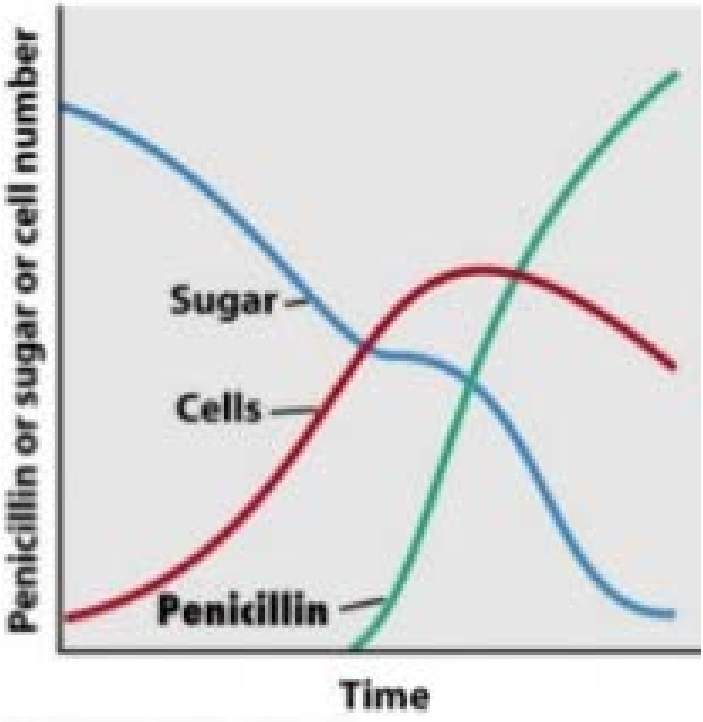
- In the early days of penicillin production, when the surface culture method was used, a variant of the original culture *of Penicillium notatum* discovered by Sir Alexander Fleming was employed.
- When however the production shifted to submerged cultivation, a strain of *Penicillium chrysogenum* designated NRRL 1951 (after Northern Regional Research Laboratory of the United States Department of Agriculture) discovered in 1943, was introduced.
- In submerged culture it gave a penicillin yield of up to 250 Oxford Units [1 Oxford Unit = 0.5988 of sodium benzyl penicillin (Penicillin G)] which was two to three times more than given by *Penicillium notatum*.
- A 'super strain' was produced from a variant of NRRL 1951 and designated X 1612.
- Present-day penicillin producing *P. chrysogenum* strains are far more highly productive than their parents. They were produced through natural selection, and mutation using ultra violet irradiation, X-irradiation or nitrogen mustard treatment.
- Present day penicillin-producing strains are highly unstable, therefore commonly stored in liquid nitrogen at – 196° or the spores may be lyophilized.

Fermentation for penicillin production

- **Inoculum** of a high yielding strain of *P. chrysogenum* is prepared as follows:
  - inoculum is first cultured in flasks in wheat bran nutrient solution for 1 week at 24°C.
  - The culture is then transferred to inoculum tanks, and grown for 1-2 days under aeration; this supports heavy mycelial growth.
  - The inoculum is now added to very large fermentors containing the production medium
- **Fermentation media** consists of 10 per cent total glucose/molasses, 4-5% corn-steep liquor solids, 0.5-0.8 per cent phenylacetic acid and 0.5% vegetable oil; pH is adjusted to 6.0 and temperature is regulated at 25- 26°C.
- Earlier media contained lactose, but it is no longer used.
- The fermentation is carried out under aerobic conditions, and nutrient supply is maintained by regular feeding (fed-batch culture). The fungus grows in a submerged culture mostly as mycelial balls.
- The fermentation is carried out for 7 days.

Fermentation kinetics

- Penicillin fermentation can be divided into three phases.
  - The first phase (trophophase) during which rapid growth occurs, lasts for about 30 hours during which mycelia are produced. Mycelial growth occurs, and carbohydrates are used up.
  - The second phase (idiophase) lasts for five to seven days; growth is reduced and penicillin is produced. Reduction of carbohydrate level in the medium favours penicillin production, which begins from the second day of fermentation. The pH of medium rises to 8.0 by the 7th day, and penicillin production stops at this stage.
  - In the third phase, carbon and nitrogen sources are depleted, antibiotic production ceases, the mycelia lyse releasing ammonia and the pH rises.



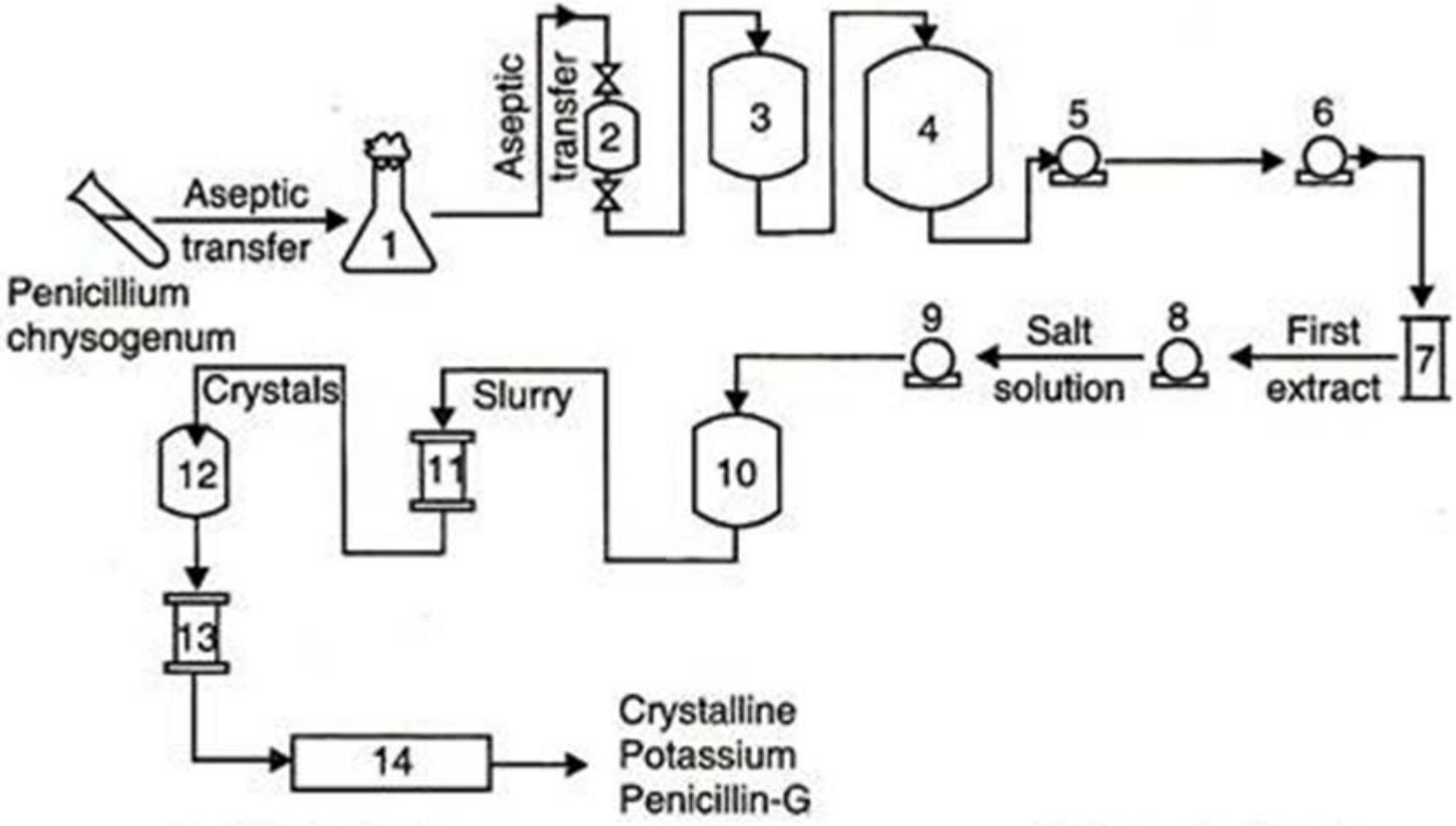
- Phenylacetic acid is the precursor for the benzene ring side chain of Penicillin G.
- The presence of precursor promotes the production of Penicillin G.

Down stream processing

- At the end of fermentation period, the fungal biomass is separated by filtration and used as animal feed supplement.
- Penicillin is highly reactive and is easily destroyed by alkali conditions (pH 7.5-8.0) or by enzymes. It is therefore cooled rapidly to 5-10°C. Since penicillins are monobasic carboxylic acids they are easily separated by solvent extraction.

Steps involve:

- The fermentation broth is filtered with a rotary vacuum filter to remove mycelia and other solids and the resulting broth is adjusted to about pH 2 using a mineral acid.
- It is then extracted with a smaller volume of an organic solvent such as amyl acetate or butyl acetate, keeping it at this very low pH for as short a time as possible.
- The aqueous phase is separated from the organic solvent.
- The organic solvent containing the penicillin is then typically passed through charcoal to remove impurities and penicillin concentrated by repeated back extraction in phosphate buffer (pH 2) and extraction in organic solvent.
- When it is sufficiently concentrated the penicillin may be converted to a stable salt form in one of several ways which employ the fact that penicillin is an acid: (a) it can be reacted with a calcium carbonate slurry to give the calcium salt which may be filtered, lyophilized or spray dried. (b) it may be reacted with sodium or potassium buffers to give the salts of these metals which can also be freeze or spray dried; (c) it may be precipitated with an organic base such as triethylamine.

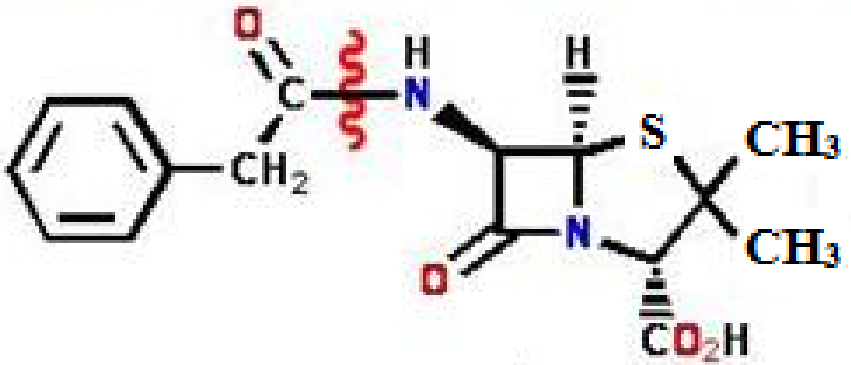


1. Shaker flask
2. Transfer vessel
3. Seed tank
4. Fermentation tank
5. Rotary filter
6. First extractor
7. Purification column
8. Second extractor
9. Third extractor
10. Vacuum still
11. Ceramic filter
12. Crystal wash
13. Ceramic filter
14. Vacuum drier

Flow sheet for large-scale production of Penicillin

Production of semi-synthetic penicillins

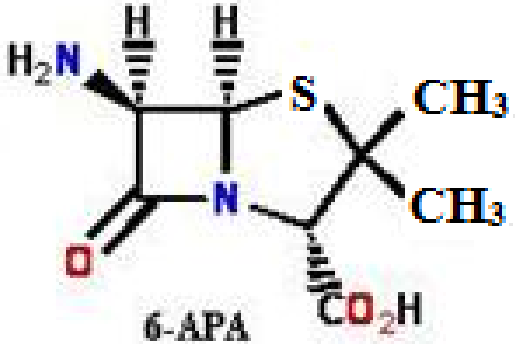
- The natural penicillins formed in unsupplemented media and the biosynthetic produced by the addition of specific side-chain precursors.
- The high expectations of making new penicillins by the introduction of side chains during fermentation, did not however, result in many new pencillins.
- In preparing semi-synthetic penicillins, 6-APA is not produced by starving *P. chrysogenum* of precursors, because yields are low.
- It is prepared by cleaving from penicillin G or penicillin V, the 6-acyl group by chemical means or with enzymes (acylases) produced by a wide range of microorganisms including bacteria, yeasts, and molds and even mammals (hog kidney acylase).
- The introduction of the acyl side chain is done by reacting 6-APA with a suitable derivative of a carboxylic acid, usually a chloride, in organic solvents under anhydrous or aqueous conditions. In the latter system it is done in acetone-water mixtures in the presence of sodium bicarbonate.
- The resulting penicillins can be extracted by solvent extraction as already described.



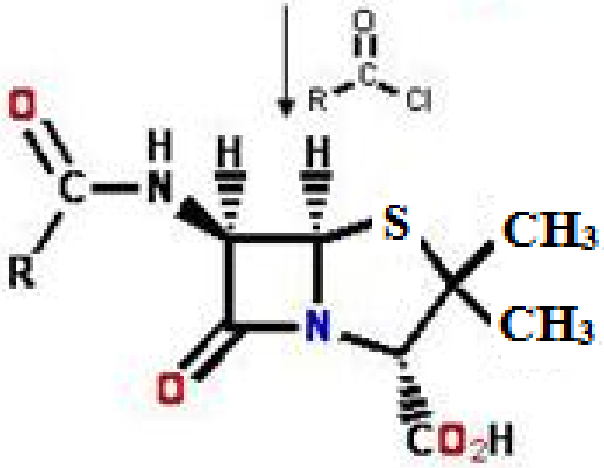
Penicillin G

Penicillin acylase

Fermentation



6-APA



Semi-synthetic penicillins

Questions

- Write an essay on industrial production of penicillin.
- Discuss the sensitivity of penicillins (β-lactams) to microbial enzymes.
- Explain fermentation kinetics of penicillins production.
- Write a short note on semi-synthetic penicillin production.
- What are penicillins and their derivatives? Explain their mode of action and classification.