

# MANUFACTURE OF ANTIBIOTICS

By:

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# PENICILLIN

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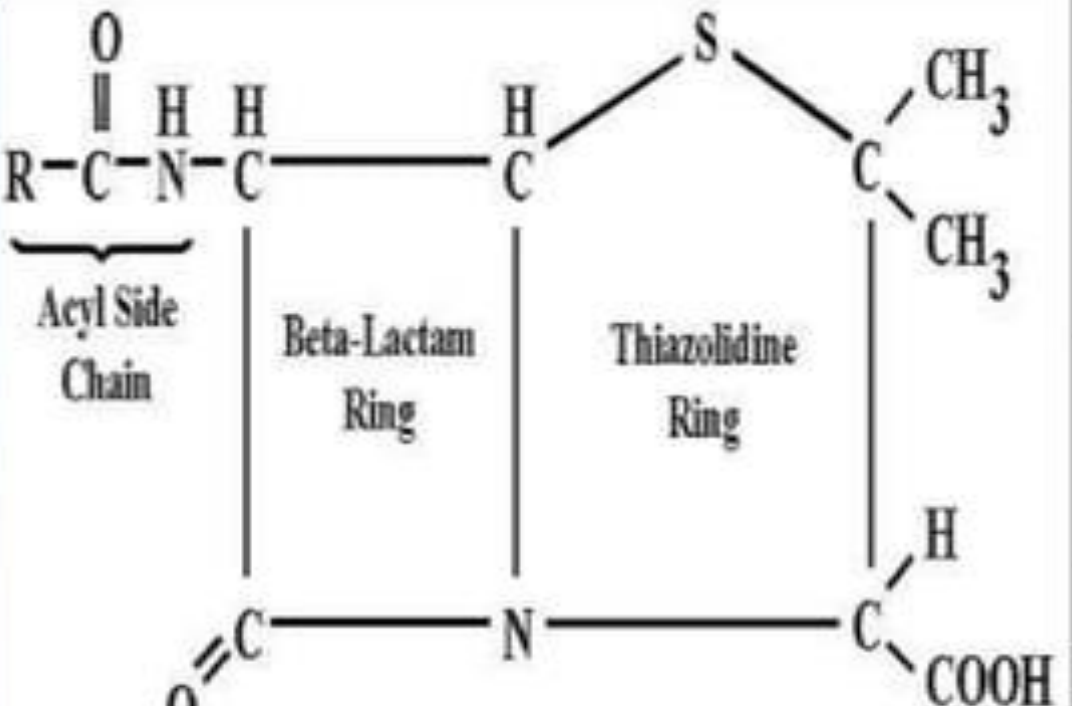
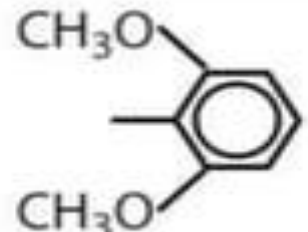
**Penicillins** are a group of  $\beta$ -lactam antibiotics obtained from *Penicillium* moulds, mainly *P. chrysogenum* and *P. rubens*. Most penicillins in clinical use are synthesised by *P. chrysogenum*.

- **First discovered Antibiotic.**
- Discovered by **Alexander Fleming** in 1928.
- **Cell wall inhibitor** – Inhibits peptidoglycan synthesis.
- Penicillin is a group of antibiotics which includes **Penicillin G, Penicillin V, Amoxillin, Ampicillin, Methicillin, Oxacillin, Dicloxacillin, Carbenicillin, Propicillin and Benzathine penicillin**
- **Narrow spectrum antibiotic**
- More effective against Gram positive bacteria.

# *Penicillium chrysogenum*



# Penicillin

| Penicillin Structure  | R Group   | Drug Name    |
|---|---|--------------|
|  <p>The diagram illustrates the general structure of penicillins, which consists of a fused bicyclic core. On the left, an <b>Acyl Side Chain</b> is attached to the <b>Beta-Lactam Ring</b> (a four-membered ring containing one nitrogen atom). This ring is fused to a <b>Thiazolidine Ring</b> (a five-membered ring containing one sulfur atom). The thiazolidine ring has two methyl groups (<math>\text{CH}_3</math>) attached to one of its carbons and a hydrogen atom (<math>\text{H}</math>) attached to another. The nitrogen atom of the beta-lactam ring is also attached to a hydrogen atom (<math>\text{H}</math>) and a carboxyl group (<math>\text{COOH}</math>).</p> <p style="text-align: center;"><b>General Structure of Penicillins</b></p> | $-\text{CH}_2-\text{C}_6\text{H}_5$   | penicillin G |
|   | $\text{CH}_2-\text{O}-\text{C}_6\text{H}_5$   | penicillin V |
|   | $-\text{CH}(\text{NH}_2)-\text{C}_6\text{H}_5$  | ampicillin   |
|   | $-\text{CH}(\text{NH}_2)-\text{C}_6\text{H}_4-\text{OH}$                              | amoxicillin  |
|   |  | methicillin  |

## **Steps Involved In Penicillin Production**

1. Selection of microorganism
2. Selection of raw materials
3. Preparation of inoculum
4. Fermentation process
5. Product recovery

# 1. Selection of Microorganisms

- Fungi mold – *Penicillium notatum* (in beginning) and *Penicillium chrysogenum* (used in present days due to high yield).

The production strain *Penicillium chrysogenum* was stored by three methods.

- i. Mixing of spore suspension with sterile inert support (soil or sand) and desiccated (extreme dryness).
- ii. Lyophilization of spore suspension (Freeze drying).
- iii. Storage of spore suspension in Liquid nitrogen in a frozen state.

## 2. Selection Of Raw Materials

- i. Abundant growth of mycelium
  - ii. Maximum accumulation of penicillin
  - iii. Ease of extraction and purification of antibiotic
- **Carbon sources** – Lactose acts as a satisfactory carbon source. Glucose and Sucrose may be used as substitutes for Lactose. Glycerol and Sorbitol are less used.
  - **Nitrogen sources** – Ammonium sulphate, Ammonium acetate and Ammonium lactate. Nitrogen is often supplied as Ammonia gas. Corn steep liquor also supplies cheap and readily available nitrogen.
  - **Mineral sources** – Potassium, Phosphorous, Magnesium, Sulphur, Iron and Copper.

### 3. Preparation Of Inoculum

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- The aim is to develop a pure inoculum in sufficient volume.
- Two types of inoculum:
  - i. Seed stage culture:** Fungal spores are collected by microchapper and inoculate in fermentation medium.
  - ii. Production stage culture:** Fungal mycelium is directly inoculated in fermenter. Liquid medium is used.



## Microbial Inoculation Methods

Three inoculation methods:

- i. Dry spores:** Inoculation of dry spores in the fermentation medium.
- ii. Suspension of spores:** A non – toxic, lauryl sulfonate or sterile water is used to make suspension. Such suspension may be applied by Spray guns or Pipettes.
- iii. Pellet inoculum:** Suspension of germinated spores, with the formation of mycelial growth at the surface of medium – pellets.

# Fermentation Medium

- The medium suggested by **Moyer and Coghill (1946)** was used for the industrial production of Penicillin.

|                                       |   |           |
|---------------------------------------|---|-----------|
| Glycerol                              | – | 7.5 g/L   |
| Cane Molasses                         | – | 7.5 g/L   |
| Corn Steep Liquor                     | – | 2.5 g/L   |
| MgSO <sub>4</sub> . 7H <sub>2</sub> O | – | 0.050 g/L |
| KH <sub>2</sub> PO <sub>4</sub>       | – | 0.060 g/L |
| Peptone                               | – | 5.0 g/L   |
| NaCl                                  | – | 4.0 g/L   |
| Iron Tartarate                        | – | 0.005 g/L |
| CuSO <sub>4</sub> . 5H <sub>2</sub> O | – | 0.004 g/L |
| Agar                                  | – | 2.50 g/L  |

## 4. Fermentation Process

Raw material / synthetic medium is added in fermenter



Inoculation of fungal mycelium



Temperature 20-24 °C



pH 6.5 - 7



Incubation 6 - 7 days

## 5. Recovery Process

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Filteration mycelial removal (vacuum filter)



Add phosphoric acid - lowers pH



Precipitation of penicillin



Broth is extracted by counter current extraction method with organic solvent (alkyl butyl acetate/ amyl acetate)

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## Recovery Process – Contd

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Back extract penicillin by adding inorganic solvent KOH / NaOH to form salt of penicillin

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Salt of penicillin

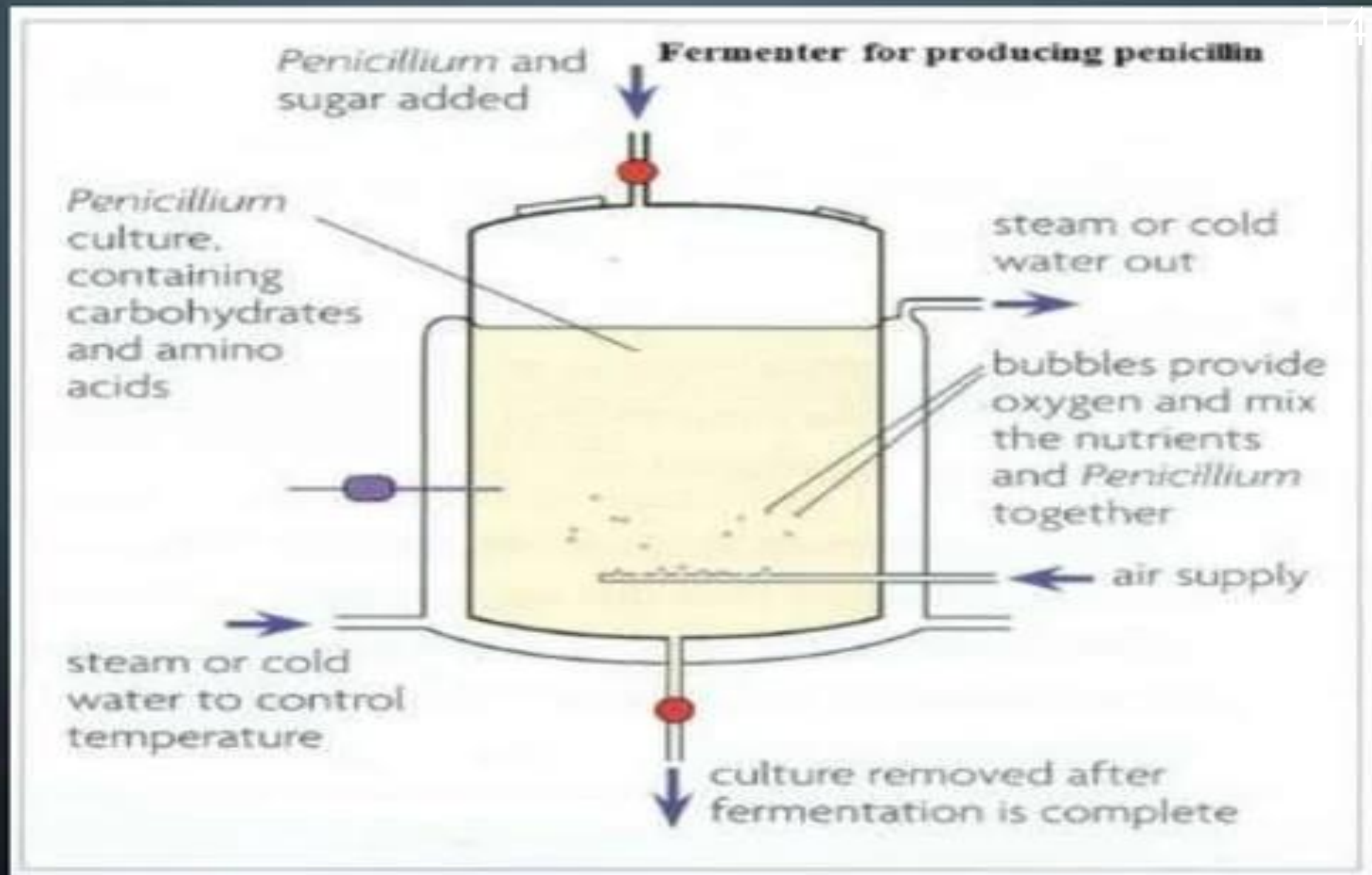


Wash, filter, dry



Chemical / enzymatic modification of penicillin

- Penicillin fermenter



# the production of penicillin.

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By the addition of different acyl donors to the medium, different penicillins can be biologically synthesized. For example, penicillin V is made by a similar process to benzylpenicillin



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but with phenoxyacetic acid as the precursor instead of PAA..  
The microorganism is again *P. chrysogenum*



## The production of cephalosporin C

It is possible to convert penicillin V or benzylpenicillin to a cephalosporin by chemical ring expansion. The first-generation cephalosporin cephalexin, for example, can be made in this way. Most cephalosporins used in clinical practice, however, are semi-synthetics produced from the fermentation product cephalosporin C.