FERMENTATIVE PRODUCTION OF PENICILLIN

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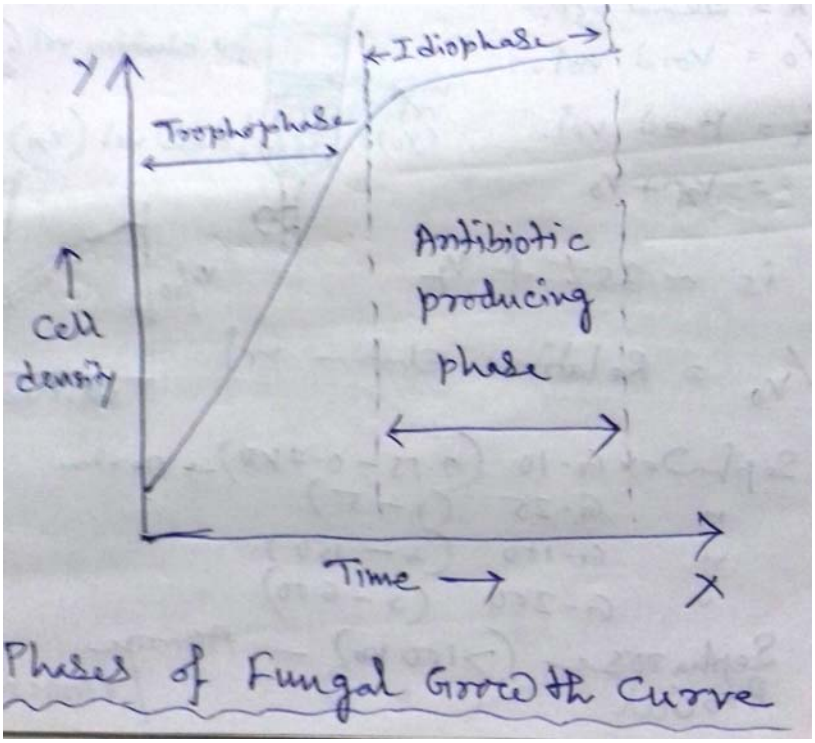
1. The Microorganisms:

The commercial organism for Penicillin fermentation is Mold *Penicillium notatum* NRRL 832 or *P. chrysogenum* NRRL 1951-B25 or *P. chrysogenum*1249-B21, however *P. chrysogenum* Q176 is the most efficient strain having highest yield of penicillin. UV irradiation of *P. chrysogenum* wild type strain resulted in the production of genetically mutated strain *P. chrysogenum* Q176. Penicillin is produced in the late phase of fungal growth (Idiophase), and hence the antibiotic is often called Idiolyte.



2. Preparation of Inoculum:

Penicillium notatum or *P. chrysogenum* are grown in solid medium for spore formation using the medium with following ingredients:

Molasses, peptone, NaCl, $CaCO_3$, $MgSO_4$, KH_2PO_4, $FeSO_4$, $CuSO_4$ & agar, pH: 6.5.

Sufficient spores may be obtained by growing the molds in large Petri dishes for 2-3 days at 25^0C. The spores are then transferred in the Roux bottle with the medium containing wheat-bran as C-source and incubated for 5-7 days at 24-26^0C. Spores are collected and washed with sterile phosphate buffer (pH: 7.0) and used as final inoculum.

3. Fermentation conditions:

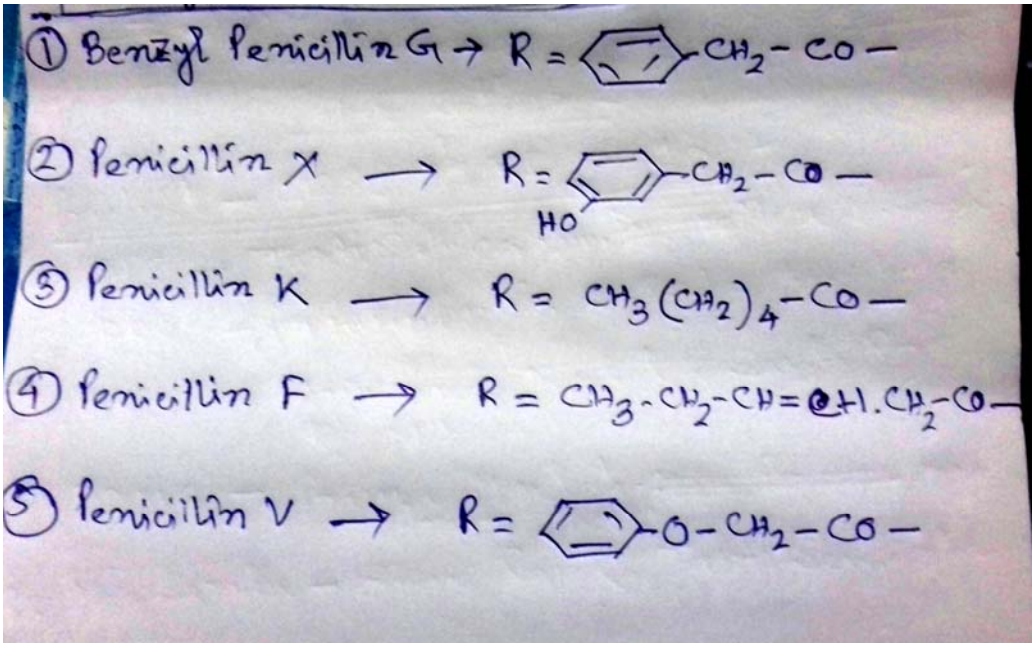
a) Nutrient source: **Lactose** is the most preferred C-source, since glucose is very rapidly utilized by the microbes, resulting in the formation of a dense mycelial network. Therefore, production rate of the antibiotic becomes low. Molasses or glycerol may be used as C-source.

b) Oxygen Concentration: Since penicillin producing microbes are highly aerobic, a large amount of oxygen must be supplied during the fermentation cycle. This is accomplished by blowing a continuous stream of sterile air in the fermentation medium. On glucose medium the rate of the growth of *Penicillium* is highest and therefore oxygen consumption is very high in glucose medium.

c) pH: **Initial pH is 6.5 – 7.0**, but the pH of the idiophase becomes alkaline (**final pH – 8.3**) due to production of NH_3. Therefore, the pH of the idiophase is carefully maintained by adding lactic acid.

d) Precursors: **Phenyl acetic acid** (PAA) should be added as precursor in the fermentation medium as **penicillin G**, the oldest form of natural penicillin, as the side chain of penicillin G contains PAA. Replacing PAA with p-nitrobenzyl acetic acid results in the production of **p-nitrobenzyl penicillin**. Similarly, **p-iodobenzyl penicillin** or **p-tolylmercaptomethyl penicillin, p-bromophenylmercaptomethyl penicillin** may be produced as semi-synthetic penicillin by altering the PAA moiety with suitable groups. Semi-synthetic penicillins are found to be more accurate for treatment of the patients (2nd generation antibiotic*) than natural penicillins like penicillin G, Penicillin K, Penicillin F or Penicillin V (1st generation antibiotic*).

[Note: Generation of antibiotic*: When antibiotic is in its native form or obtained from microbes with no alternation, it is called 1st generation antibiotic. After several application of this antibiotic to the target organisms may develop resistance against the antibiotic. Then some alternations are created in the structure of the native antibiotic to make it effective against the target microbes. Such modified antibiotic is called 2nd generation antibiotic. If repeated use again develop resistance to that antibiotic then alternation of its structure is done again to make it effective. Such structurally altered antibiotic is termed as 3rd generation antibiotic and so on.]



4. Fermentation:

There are two types of fermentation applied for the industrial fermentation of penicillin.

a) Surface culture fermentation: The method is based on the natural tendency of molds to grow as a thin layer on the surface of liquid media. The fundamental requirement of this fermentation is the depth of the medium should be app. 2 cm. Dry spores of the fungi are suspended to the surface of the medium. Lactose is the chief carbon source. The medium is incubated for 8 days at 24^0C. Initial pH is 6.8, after 5 days pH is 7.1 and after 8 days pH is 8.2. Maximum production of the antibiotic occurs in between 6^{th} -7^{th} day. Drawback of the method is it is expensive, laborious and economically impractical and therefore this method is no longer used now-a-days. Maximum yield is 193 units of penicillin/ml of medium (on 7^{th} day of fermentation).

b) Submerged fermentation: In this process, spores are homogenously suspended through the liquid fermentation medium. Fermentation medium consist of lactose, cerelose, corn steep liquor, $NaNO_3$, KH_2PO_4, $ZnSO_4$, $CaCO_3$, and phenyl acetic acid. The medium is sterilized in the fermentation flask for 30 minutes at 15 psi steam pressure. Fermentation takes 5-7 days for completion. Fermentation flasks are incubated at 24^0C. Initial pH is 6.8, and final pH is 8.3 (on 7^{th} day of fermentation). Maximum yield occurs on the 7^{th} day of fermentation (560 units of penicillin/ ml of medium).

5. Down-stream processing:

After the fermentation is completed, the medium is filtered through a filter bed to separate mycelium from the fermented broth. The filtrate is taken and acetone is added to it. The antibiotic comes to the acetone layer. Acetone layer is carefully transferred in another fresh bottle and allowed to evaporate acetone. The antibiotic is washed twice again with acetone to remove impurities associated with the antibiotic.

6. Uses:

Na- salt of benzyl penicillin (Penicillin G), better known as procaine penicillin, used as antibiotic injection to treat bacterial infections, specially caused by Gram positive bacteria.