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Article

Features of Industrial Production of Penicillin: From Laboratory Discovery to Biotechnological Production

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Abstract: Penicillin is the first antibiotic introduced into broad clinical practice, the history of which became the starting point of the era of antimicrobial chemotherapy. However, the path from Alexander Fleming's accidental observation to the powerful industry of fermentation production took more than a decade and required solving extremely complex biochemical, technological, and engineering problems. This work examines the key features of penicillin biosynthesis, the stages of selection of productive Penicillium chrysogenum strains, the parameters of deep cultivation, and the principal scheme for obtaining semi-synthetic analogues of natural antibiotics. Special attention has been paid to the role of the penicillinase enzyme in the formation of 6-aminopenicillinic acid - a universal "building block" for modern modified penicillins.

Keywords: Penicillium chrysogenum, benzylpenicillin, fermentation, 6-aminopenicillin acid, penicillin acylase, biosynthesis, deep cultivation, plant mycelium.

Introduction

The history of penicillin is unique not only in the very fact of its discovery, but also in the unprecedented pace of transformation of the laboratory phenomenon into large-scale production. In 1929, Alexander Fleming, while working at St. Mary's Hospital in London, noticed the lysis of staphylococcal colonies in a Petri dish accidentally infected with mold fungi. However, nine years passed before the Oxford group led by Howard Florey and Ernst Chain managed to isolate penicillin in crystalline form. By 1943, at the height of World War II, the first industrial line was launched, saving hundreds of thousands of lives and forever changing the face of medicine. In 1945, the Nobel Committee rightfully recognized the merits of the triumvirate - Fleming, Florey, and Chain[1].

Today, eight decades later, penicillin remains not only a historical symbol but also a real production asset. The scale and effectiveness have fundamentally changed: if the first industrial strains yielded no more than 20 units of activity per milliliter (approximately 12 μg/ml), then modern producers are capable of synthesizing more than 15,000-25,000 units/ml. This thousand-fold growth is the result of targeted selection, optimization of environments and cultivation regimes[2].

Biological basis and taxonomy of producers

The ability to synthesize penicillin is common among many representatives of the Aspergillus and Penicillium genera, however, only strains belonging to the Penicillium notatum and, especially, Penicillium chrysogenum groups have industrial significance. It was the latter species that became the object of in-depth selection work[3].

Natural isolates isolated from soil substrates had extremely low productivity. Systematic work on the selection of highly active variants, induced mutagenesis, and optimization of cultivation conditions has allowed for the production of strains whose productivity is three orders of magnitude higher than that of wild predecessors. It is important to emphasize: it is these modified lines that are used today in industry, while the original cultures retain only museum significance[4].

Chemical identification and classification

The term "penicillin" encompasses an extensive group of compounds representing acyl derivatives of a heterocyclic amino acid - 6-aminopenicillinic acid. Natural representatives are benzylpenicillin (penicillin G) and phenoxymethylpenicillin (penicillin V). It is they that serve as the initial raw material for further chemical modifications[5].

Research Methodology

This study is based on theoretical and analytical research methods aimed at examining the technological and biological aspects of industrial penicillin production. The main methodological approach used in the research is the analysis and systematization of scientific literature related to microbiology, biotechnology, and industrial antibiotic production.

A comprehensive literature review was conducted to analyze the historical development of penicillin production, beginning from its laboratory discovery to the establishment of large-scale industrial fermentation processes. Scientific textbooks, research articles, and academic publications were studied to identify the biological characteristics of Penicillium chrysogenum and the technological factors influencing penicillin biosynthesis.

In addition, a comparative analytical method was applied to evaluate the effectiveness of different cultivation conditions, including temperature regimes, oxygen supply, nutrient composition, and pH levels used during deep fermentation. Particular attention was given to the preparation of inoculum material and the role of nutrient media containing carbon, nitrogen, and mineral components necessary for the growth of the producing microorganism.

The research also considers biochemical mechanisms involved in the formation of semi-synthetic penicillins through enzymatic processes, including the role of penicillin acylase in the formation of 6-aminopenicillinic acid (6-APA). The obtained information was analyzed and summarized to describe the modern technological principles of industrial penicillin production.

Results and Discussion

Technological parameters of deep cultivation

Penicillin biosynthesis is a strictly aerobic process. The need of mycelial fungi for oxygen dictates the need for intensive aeration and mixing of the culture liquid. The optimal range for oxygen supply ranges from 0.38 to 4.38 g O2/ (l·h). The critical parameter is temperature. There are two possible modes[6]:

1. Classic low-temperature (28 °C) - maximum biosynthesis is achieved in the first 24 hours, however, the absolute values of antibiotic accumulation lag behind the alternative mode[7].

2. Increased temperature (40 °C) - peak production occurs between 24-48 hours, while the total penicillin yield is significantly higher[8].

1.The regulation of the acidity of the medium is carried out at the stage of isolating the target product. For example, when obtaining gramicidin C, the culture liquid is acidified with hydrochloric acid to pH 4.5-5.0, causing the precipitation of gramicidin dichlorohydrate along with the producer's biomass. Further extraction of the antibiotic is carried out with ethyl alcohol to obtain a 4% concentrate[9].

2.Semi-synthetic penicillins: second generation biotechnology

3.A true breakthrough was the mastery of the semi-synthetic pathway, combining biological fermentation with chemical modification. This strategy includes two mandatory stages:

Biosynthesis and isolation of a culture liquid containing penicillin in the form of a free acid. There are two possible approaches:

o fermentation with specific conditions for the development of P. chrysogenum without adding a precursor;

o enzymatic deacylation of benzylpenicillin or phenoxymethylpenicillin under the influence of penicillin acylase.

4. Obtaining 6-aminopenicillinic acid (6-APC) - a key intermediate product practically devoid of antibacterial activity (activity ratio to benzylpenicillin is 1:2000), but serving as a platform for the synthesis of modified penicillins.

The possibility of 6-APC biosynthesis was first noted by Kalo in 1953. Later, Batchelor et al. (1959) demonstrated the isolation of 6-APK directly from the culture fluid of P. chrysogenum when cultivated in a medium devoid of precursors.

The enzymatic hydrolysis of benzylpenicillin is carried out by penicillin acylase, which breaks the carboxylamide bond between 6-APK and phenylacetic acid. It's important to distinguish:

• penicillin acylase - an enzyme that hydrolyzes the amide bond while preserving the β-lactam cycle;

• penicillinase (β-lactamase) - an enzyme that breaks down the β-lactam ring to form biologically inactive penicilloic acid.

Penicillinase is widespread among microorganisms: it is produced by all fungi - penicillin producers, as well as many bacteria (Escherichia coli, Actinomyces lavendulae, Nocardia) and yeasts. The enzyme of bacterial origin exhibits maximum activity in relation to benzylpenicillin G, while the enzymes of eukaryotic producers more actively hydrolyze other natural forms of penicillin[10].

A study of E. coli strains showed that 79% synthesize penicillinase, 72% - acylase, and 59% produce both enzymes simultaneously. Only 6% of cultures do not exhibit enzymatic activity against penicillins.

Sowing material preparation: the foundation of productive fermentation

Inoculate quality is a critical factor determining the effectiveness of the entire technological cycle. Sowing material preparation goes through two stages:

1. Growing first-generation mycelium in small-volume inoculators.

2. Cultivation of second-generation seed mycelium in machines with increased capacity.

The initial spore culture is propagated on sterile millet in glass bottles, followed by drying and storage at room temperature. Inoculation is carried out with dry spores from 2-3 vials.

The principal task of the first stage is to accumulate physiologically active biomass as quickly as possible, capable of ensuring intensive growth and high yield of the antibiotic when transferred to the fermenter. This is achieved by using rich media containing easily digestible carbon and nitrogen sources, combined with optimal aeration and temperature (24-26 °C)[11].

Carbon nutrition. The main source is glucose and sucrose. Lactose is introduced as a second carbohydrate component in relatively small amounts. Its presence is necessary for the adaptation of the mycelium to the fermentation of poorly digestible lactose: during the adaptation period, the synthesis of corresponding hydrolases is induced, which subsequently accelerates the consumption of this sugar and positively affects the biosynthesis of penicillin[12].

Nitrogen nutrition. The fungus's nitrogen requirement can be met by both mineral sources (ammonium and nitrate salts) and organic components. In industrial environments, corn extract - a complex raw material containing organic nitrogen, amino acids, polypeptides, vitamins, growth factors, and additional carbon - is widely used[13].

Mineral elements and microelements. For normal growth and biosynthesis, sulfur, potassium, phosphorus, magnesium, as well as microelements (manganese, zinc, iron, copper) are necessary. The typical composition of the sowing medium is presented in the table 1.

Table 1. Recipe for a medium for growing Penicillium chrysogenum seed material[14].

Component	Content, %
Corn extract (calculated as dry matter)	2.0
Glucose	2.0
Lactose	0.5
Ammonium nitrate	0.125
Monopotassium phosphate	0.2
Magnesium sulfate	0.025

Sodium sulfate	0.05
Chalk (calcium carbonate)	0.5

Medium acidity. The optimal pH values are in the range of 6.0-6.5. Shifting to the acidic or alkaline zone slows down growth processes.

Cultivation duration and conditions. The cultivation of the first generation of seed mycelium takes 36-50 hours (until the biomass reaches medium viscosity). The crop is transferred to sowing apparatuses in the amount of 10% of the volume, where it is grown for another 12-18 hours. Then 15-20% of the mature second-generation mycelium volume is transferred to large fermenters. At all stages, constant mixing and supply of sterile air in a ratio of 1:1.2-1.5 (the volume of the medium to the volume of air supplied per minute) are maintained[15].

Conclusion

The industrial production of penicillin is a classic example of a well-developed biotechnological process where fundamental knowledge of microorganism physiology, engineering solutions in the field of deep cultivation, and subtle chemical synthesis are intertwined. The evolution of production - from single laboratory installations to giant fermentation lines - became possible due to systematic selection of strains, optimization of fermentation environments and regimes, and implementation of semi-synthetic approaches.

Mastering the technology for obtaining 6-aminopenicillinic acid has opened the era of modified penicillins resistant to acidic stomach environments and bacterial β-lactamases. Today, the biosynthetic potential of Penicillium chrysogenum strains is approaching the theoretical limit, however, work continues on improving individual stages - inoculum preparation, nutrient media composition, aeration regimes - in the direction of increasing economic efficiency and environmental safety of production.

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