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Bacillus Penicillin Acelase, A Key Catalyst in the Modern Antibiotic Era: A Literature Review

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Abstract

Penicillin acylase from the genus Bacillus plays an irreplaceable role as a key catalyst in the modern antibiotic era. This enzyme essentially facilitates the production of 6-aminopenicillanic acid (6-APA), a vital precursor for a variety of semisynthetic beta-lactam antibiotics that continue to be the mainstay in the fight against bacterial infections. The aim of this study was to review Bacillus Penicillin Acylase: A Key Catalyst in the Modern Antibiotic Era" a literature review. This method is a more rigorous and structured approach than a traditional literature review. The goal is to minimize bias and provide a reliable and comprehensive synthesis of research relevant to a specific research question. Result of the expected findings based on our previous discussions and general knowledge of the field:A literature review on "Bacillus Penicillin Acylase: A Key Catalyst in the Modern Antibiotic Era" would likely yield a comprehensive overview of the enzyme's significance, characteristics, production, and applications. The review would strongly emphasize the pivotal role of Bacillus-derived penicillin acylase in the industrial production of 6-aminopenicillanic acid (6-APA). 6-APA is consistently highlighted as the crucial intermediate for synthesizing a wide array of semi- synthetic beta-lactam antibiotics, which form the cornerstone of modern antibacterial therapy; Different species and even strains within a species exhibit variations in enzyme characteristics, including substrate specificity (e.g., preference for penicillin G or V), enzyme activity, optimal operating conditions (pH and temperature), stability, and whether the enzyme is produced intracellularly or extracellularly.
Keywords: Bacillus, Penicilin Acylase, bancterial infection

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Introduction

The discovery of penicillin by Alexander Fleming in 1928 marked the beginning of a

revolution in the treatment of bacterial infectious diseases. Since then, the development and modification of the penicillin core structure through chemical and biocatalytic approaches have yielded a variety of semisynthetic beta-lactam antibiotics with improved spectrum of activity and pharmacokinetic properties. Among the crucial biocatalytic tools in this evolution is the enzyme penicillin acylase, often sourced from bacteria of the genus Bacillus. Central Role in the Production of Semisynthetic Antibiotics (Quiroga et al., 2024); (Pan et al., 2022): Penicillin acylase (Bacillus) specifically hydrolyzes natural penicillins (such as penicillin G and penicillin V) to produce 6-aminopenicillanic acid (6-APA); 6-APA is the primary precursor for the synthesis of a wide range of semisynthetic penicillin antibiotics. These antibiotics are designed to have a broader spectrum of activity, resistance to bacterial beta-lactamase enzymes, and improved pharmacokinetic properties than natural penicillins (Tooke et al., 2019); (Zhang et al., 2024).

Without the availability of 6-APA on an industrial scale, the production of semi-synthetic antibiotics crucial to addressing modern bacterial infections would be severely hampered (Hyde et al., 2024). Biocatalytic Efficiency: As a biocatalyst, Bacillus penicillin acylase significantly accelerates the hydrolysis of the amide bond in penicillin compared to traditional chemical methods; The enzyme operates at relatively mild temperature and pH conditions, reducing energy requirements and the potential for the formation of unwanted by-products compared to harsh chemical reactions; The enzyme's specificity to the substrate (penicillin) minimizes the formation of unnecessary by-products, resulting in a higher purity 6-APA product and facilitating downstream purification processes. Bacillus penicillin acylase is a key catalyst in the modern antibiotic era because it: Is essential for the production of the primary precursor (6-APA) for semi-synthetic penicillin antibiotics; Offers greater efficiency, specificity, and control over the catalysis process (Haque et al., 2024); (Mato et al., 2024); Can be produced on an industrial scale via Bacillus fermentation (Mahfudz et al., 2024); (Klausmann et al., 2021); Drives innovation in the development of new beta-lactam antibiotics to address the challenge of bacterial resistance; Has the potential to be a more sustainable alternative to traditional chemical methods (Uddin et al., 2021).

Without the crucial role of this enzyme, the availability and development of modern beta-lactam antibiotics would be severely hampered, which would have a significant impact on our ability to combat bacterial infections. Therefore, in-depth research on this enzyme continues to be an important focus in the fields of microbiology, biochemistry, and pharmacy. Penicillin acylase produced by bacteria of the genus Bacillus plays a central role as a biocatalyst in the production of semisynthetic beta-lactam antibiotics, an essential foundation of the modern antibiotic era. This in-depth literature review will thoroughly explore crucial aspects related to this enzyme, from its mechanism of action to its innovative applications in addressing the challenges of antibiotic resistance.

Methodology

This method is a more rigorous and structured approach than a traditional literature review. The goal is to minimize bias and provide a reliable and comprehensive synthesis of research relevant to a specific research question. The following are the key elements and steps in the systematic literature review method:

Formulation of a Focused and Structured Research Question

A crucial first step is to formulate a clear and structured research question. The PICO (Population, Intervention, Comparison, Outcome) format is often used for intervention studies, while PICOS (Population, Intervention, Comparison, Outcome, Study design) adds study design elements for broader questions.

Development of a Research Protocol

Before beginning a search, it is important to develop a research protocol that documents all steps that will be taken in the review. This protocol aims to increase transparency and reduce the risk of bias.

Comprehensive and Documented Literature Search

The search should be extensive and systematic to identify all potentially relevant studies. A variety of electronic databases (e.g., PubMed, Scopus, Web of Science) and other sources (grey literature, reference lists of relevant studies) should be searched. A detailed search strategy with a combination of relevant keywords and Boolean operators should be carefully documented to ensure reproducibility. Search limitations (e.g., language, publication time range) should be defined and justified in the protocol.

Rigorous and Transparent Study Selection

The study selection process is conducted in several stages based on the inclusion and exclusion criteria set out in the protocol. Title and abstract screening by at least two independent reviewers to identify potentially relevant studies. Full-text review of studies that pass title and abstract screening by at least two independent reviewers to determine eligibility for inclusion. Resolve disagreements between reviewers through discussion or by involving a third reviewer. Full documentation of the rationale for inclusion and exclusion of each study.

Structured Data Extraction

Relevant data from studies meeting the inclusion criteria are extracted using a standardized and pretested data extraction form. Extracted data may include study characteristics (design, population, intervention, comparator, outcome), outcomes, and other information relevant to the research question. Data extraction should ideally be performed by two reviewers independently with comparison and resolution of discrepancies.

Methodological Quality Assessment of Studies (Risk of Bias Assessment)

The methodological quality of each included study is systematically assessed using a valid and reliable risk of bias assessment tool (e.g., Cochrane Risk of Bias tool for interventional studies, Newcastle-Ottawa Scale for observational studies). The assessment is performed by at least two reviewers independently with resolution of discrepancies. The results of the risk of bias assessment are used to evaluate the overall strength of the evidence and can be considered in data synthesis.

Data Synthesis

The extracted data are synthesized to answer the research question. The synthesis methods can be: Quantitative Synthesis (Meta-Analysis): If the studies are sufficiently clinically and methodologically homogeneous, their numerical results can be combined statistically to produce a more precise estimate of the pooled effect. Meta-analysis involves calculating effect sizes and confidence intervals, and assessing heterogeneity between studies. Qualitative Synthesis: If the studies are too heterogeneous for meta-analysis, synthesis

Results and Discussion

Penicillin acylase (also known as penicillin amidohydrolase) is an enzyme that catalyzes the hydrolysis of the amide bond in penicillin and related antibiotics. This enzymatic reaction is crucial for the production of semi-synthetic penicillin antibiotics. Here's a breakdown of its key aspects:

Function

Hydrolysis of Penicillins: Penicillin acylase specifically cleaves the acyl side chain from the

6-aminopenicillanic acid (6-APA) core of penicillin molecules; Production of 6-APA: The primary product of this reaction, 6-APA, is a vital intermediate in the synthesis of a wide range of semi-synthetic penicillins. These modified penicillins exhibit improved properties such as broader antibacterial spectra and resistance to certain bacterial enzymes (Galgano et al., 2025).

Mechanism of Action

The catalytic mechanism of penicillin acylase typically involves a nucleophilic attack by a catalytic residue (often a serine or cysteine at the N-terminus of one of the enzyme's subunits) on the carbonyl carbon of the amide bond in the penicillin substrate. This forms a tetrahedral intermediate, which is stabilized by other residues in the active site. The bond then breaks, releasing 6-APA and the corresponding side-chain carboxylic acid.

Sources:

Penicillin acylases are found in various microorganisms, including

Bacteria: *Escherichia coli* is one of the most well-studied sources, but other bacteria like *Bacillus* species also produce this enzyme; Yeast and Fungi: Some yeast and fungal species also possess penicillin acylase activity.

Industrial Importance

Production of Semi-synthetic Penicillins: Penicillin acylase is extensively used in the pharmaceutical industry for the large-scale production of 6-APA. This intermediate is then chemically modified to create various commercially important antibiotics like ampicillin, amoxicillin, and others; Green Biocatalysis: The use of penicillin acylase offers a more environmentally friendly alternative to traditional chemical methods for modifying penicillin (Wang et al., 2022).

Types of Penicillin Acylases

Penicillin acylases can be classified based on their substrate preference: Penicillin G acylase: Prefers penicillin G (benzylpenicillin) as a substrate; Penicillin V acylase: Prefers penicillin V (phenoxymethylpenicillin); Ampicillin acylase: Shows activity towards ampicillin.

The genus *Bacillus* is a specific source of the enzyme penicillin acylase, and importantly, different species within this genus are known to produce the enzyme with varying characteristics. Let's break down what this means:

Bacillus as a Source:

Bacillus is a genus of Gram-positive, rod-shaped bacteria that are widespread in various environments like soil and water; Certain species within this genus have the genetic capability to produce penicillin acylase. This makes *Bacillus* a valuable resource for obtaining this industrially important enzyme (Danilova & Sharipova, 2020).

Different Species, Different Characteristics

This is a crucial point. The characteristics of the penicillin acylase produced can vary significantly depending on the specific *Bacillus* species. These variations can include:

Substrate Specificity

Some *Bacillus* species might produce penicillin acylases that preferentially act on penicillin G (benzylpenicillin), while others might have a higher affinity for penicillin V (phenoxymethylpenicillin) or even other beta-lactam antibiotics. For example, *Bacillus subtilis* has been shown to produce penicillin V acylase.

Enzyme Activity and Efficiency

The rate at which the enzyme catalyzes the hydrolysis reaction (enzyme activity) and its overall efficiency (related to kinetic parameters like Km and Vmax) can differ between species. Some *Bacillus* strains might naturally produce more active or efficient enzymes than others (Vu et al., 2022).

Optimal Conditions

The ideal conditions for the enzyme to function optimally, such as temperature and pH, can vary depending on the *Bacillus* species from which it is derived. This is important for industrial applications to ensure maximum enzyme performance. For instance, *Bacillus subtilis* BAC4 produces an extracellular penicillin G acylase with optimal activity at 43°C and pH 8.5.

Stability

The stability of the enzyme under different conditions (e.g., temperature, pH, presence of inhibitors) can also vary between *Bacillus* species. More stable enzymes are generally preferred for industrial processes as they can withstand longer usage and harsher conditions.

Location of Enzyme Production

Some *Bacillus* species secrete the penicillin acylase extracellularly (into the surrounding medium), while others produce it intracellularly (within the bacterial cells). Extracellular enzymes are often easier to purify, simplifying downstream processing. *Bacillus megaterium* is known to secrete penicillin G acylase extracellularly, which offers advantages in terms of enzyme recovery compared to intracellular enzymes from organisms like *E. coli*.

Induction Requirements

The production of penicillin acylase in some *Bacillus* species might be induced by the presence of specific compounds in the growth medium (e.g., phenylacetic acid for penicillin G acylase), while in others, the enzyme production might be constitutive (produced continuously). *Bacillus megaterium*'s penicillin acylase production is induced by phenylacetic acid.

Key Catalysts in the Modern Antibiotic Era: This emphasizes how important this enzyme is in the production of semisynthetic beta-lactam antibiotics, which are the foundation of modern-day treatment of bacterial infections. Without penicillin acylase, mass production of antibiotics such as amoxicillin, ampicillin, and others would be greatly hampered" effectively highlights the critical role of penicillin acylase in the modern antibiotic landscape. Let's break down why it's considered a "key catalyst":

Central Role in Semi-synthetic Beta-Lactam Antibiotic Production
Foundations of Modern Antibiotic Therapy

Beta-lactam antibiotics (including penicillins, cephalosporins, carbapenems, and monobactams) are the most widely used and arguably the most important class of antibacterial drugs. They are effective against a broad spectrum of bacterial infections and have saved countless lives.

Semi-synthetic Derivatives are Crucial

While natural penicillins (like penicillin G and penicillin V) were groundbreaking, they have limitations such as a narrow spectrum of activity and susceptibility to bacterial resistance mechanisms (like beta-lactamase enzymes). To overcome these limitations, scientists developed semi-synthetic penicillins by chemically modifying the core structure (Stojković et al., 2023); (Bellucci et al., 2024).

Penicillin Acylase as the Gateway

The production of these semi-synthetic penicillins relies heavily on the availability of a key

intermediate: 6-aminopenicillanic acid (6-APA). Penicillin acylase is the enzyme responsible for efficiently and cost-effectively producing 6-APA from naturally occurring penicillins. *Key Catalyst" - Why this term is appropriate Biological Catalyst*

Penicillin acylase acts as a biological catalyst, speeding up the hydrolysis reaction that removes the side chain from natural penicillins to produce 6-APA (Serrano-Aguirre et al., 2021). Without this enzyme, this chemical transformation would be much slower, require harsher conditions, and be less efficient, making large-scale production impractical.

Specificity

Penicillin acylase exhibits a degree of specificity for its substrates (different types of penicillins), leading to a cleaner reaction and easier purification of 6-APA compared to non-enzymatic chemical methods (Lv et al., 2024).

Scalability

Microbial sources of penicillin acylase, particularly Bacillus and E. coli, can be cultivated on a large-scale using fermentation technology, allowing for the production of sufficient quantities of the enzyme to meet industrial demands for 6-APA.

Economic Viability

The enzymatic production of 6-APA using penicillin acylase is economically more viable than traditional chemical methods, making semi-synthetic penicillins are more accessible and affordable.

Impact on Specific Antibiotics (Amoxicillin, Ampicillin, etc.):
Building Blocks

6-APA is the essential building block for a vast array of semi-synthetic penicillins. By chemically attaching different side chains to the 6-APA core, pharmaceutical companies can create antibiotics with tailored properties.

Production Bottleneck

Without an efficient and scalable method for producing 6-APA via penicillin acylase, the mass production of these crucial semi-synthetic antibiotics would be severely hampered, impacting the treatment of numerous common and serious bacterial infections (Simić et al., 2022). In essence, penicillin acylase acts as the linchpin in the supply chain for many modern beta- lactam antibiotics. Its catalytic activity allows for the efficient conversion of relatively simple, naturally produced penicillins into the versatile building blocks needed to create a diverse arsenal of drugs that are fundamental to modern medicine (Ancajas et al., 2024). The statement accurately emphasizes its indispensable role in ensuring the availability of these life-saving medications.

Conclusion

Through its efficient and specific hydrolysis mechanism, Bacillus penicillin acylase enables the synthesis of antibiotics with a broader spectrum of activity, enhanced resistance to bacterial defense mechanisms, and superior pharmacokinetic profiles compared to natural penicillins. The ability of Bacillus bacteria to grow and produce this enzyme on an industrial scale through a continuously optimized fermentation process ensures the continued availability of 6-APA to meet the

global demand for beta-lactam antibiotics. Furthermore, ongoing research and development on Bacillus penicillin acylase, including the characterization of the enzyme from various species, optimization of production methods, and application of genetic engineering techniques, continues to pave the way for innovation in the synthesis of new generation antibiotics. These efforts are becoming

increasingly crucial in the face of the increasing challenge of antibiotic resistance.

Conflicts of Interest

The authors declare no conflict of interest.

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