International Journal of Pharmacy and Pharmaceutical Science
www.pharmacyjournal.org
Online ISSN: 2664-7230; Print ISSN: 2664-7222; Impact Factor: RJIF 5.44
Received: 02-04-2020; Accepted: 11-05-2020; Published: 14-06-2020
Volume 2; Issue 2; 2020; Page No. 01-04

Microbial production of vitamins and amino acids

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DOI: https://doi.org/10.33545/26647222.2020.v2.i2a.98

Abstract
Microbial production of vitamins and amino acids has emerged as a sustainable and efficient alternative to traditional chemical synthesis. Microorganisms such as bacteria, yeast, and fungi are harnessed for their metabolic capabilities to produce these essential nutrients. This research article reviews the current advancements in microbial production of vitamins and amino acids, discussing the underlying metabolic pathways, genetic engineering strategies, and bioprocess optimization techniques. The potential applications and future prospects of microbial production in the nutraceutical and pharmaceutical industries are also explored.

Keywords: Amino acids, microbial production, vitamins

Introduction
Vitamins and amino acids are essential nutrients required for various physiological functions in humans and animals. Traditionally, these compounds have been produced through chemical synthesis, which often involves complex processes and harsh conditions, leading to environmental concerns. Microbial production, leveraging the metabolic capabilities of microorganisms, offers a more sustainable and cost-effective alternative. Microorganisms such as bacteria, yeast, and fungi can be engineered to overproduce these nutrients, providing a renewable source of vitamins and amino acids.
The metabolic pathways involved in the biosynthesis of vitamins and amino acids in micro-organisms are well-characterized. Advances in genetic engineering have enabled the modification of these pathways to enhance production yields. Furthermore, bioprocess optimization techniques, including fermentation process control and metabolic flux analysis, have significantly improved the efficiency of microbial production systems. This article provides a comprehensive overview of the microbial production of key vitamins and amino acids, highlighting the latest developments and future prospects.

Objective
The objective of this research article is to review and analyze the advancements in microbial production of vitamins and amino acids, focusing on the underlying metabolic pathways, genetic engineering strategies, and bioprocess optimization techniques, and to explore their potential applications and future prospects in the nutraceutical and pharmaceutical industries.

Metabolic Pathways in Microbial Production
Microorganisms synthesize vitamins and amino acids through intricate and highly regulated metabolic pathways. These pathways are optimized for the production of essential nutrients and are subject to sophisticated genetic and enzymatic controls. Understanding these pathways is crucial for harnessing microorganisms for industrial production.

Vitamins
The production of vitamins in microorganisms involves several distinct biosynthetic pathways, each tailored to the specific vitamin.
Riboflavin (Vitamin B2) is synthesized via the riboflavin biosynthesis pathway. This pathway begins with the precursor molecules guanosine triphosphate (GTP) and ribulose-5-phosphate. Through a series of enzymatic reactions, these precursors are converted into riboflavin. Key enzymes in this pathway include GTP cyclohydrolase II, which catalyzes the conversion of GTP to 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate, and riboflavin synthase, which catalyzes the final step converting 6,7-dimethyl-8-ribityllumazine to riboflavin. Microorganisms such as Corynebacterium glutamicum and Bacillus subtilis are commonly engineered to overproduce riboflavin by enhancing the expression of these key enzymes and by removing regulatory controls that limit production.
Vitamin C (Ascorbic Acid) production in microorganisms typically involves the conversion of glucose to ascorbic acid through intermediate compounds. For instance, in the case of Gluconobacter oxydans, the pathway includes the oxidation of D-sorbitol to L-sorbose, followed by its conversion to 2-keto-L-gulonic acid (2-KGA), which is then transformed into L-ascorbic acid. The critical enzymes in this pathway are sorbitol dehydrogenase, which oxidizes D-sorbitol to L-sorbose, and sorbosone dehydrogenase, which further converts L-sorbose to 2-KGA. Genetic engineering efforts focus on optimizing the activity of these enzymes and increasing the flux through the pathway to enhance vitamin C yields.

Amino Acids
Amino acids are produced through various biosynthetic pathways in microorganisms, each involving multiple steps and precise regulation.

Lysine is synthesized via the diaminopimelate (DAP) pathway in microorganisms such as Corynebacterium glutamicum. This pathway begins with the precursor molecule aspartate, which is converted to dihydrodipicolinate through a series of enzymatic steps. Key enzymes include aspartokinase, which catalyzes the initial phosphorylation of aspartate, and dihydrodipicolinate synthase, which catalyzes the formation of dihydrodipicolinate. The pathway culminates in the conversion of diaminopimelate to lysine. Genetic modifications to enhance lysine production often involve overexpressing these key enzymes, deleting genes responsible for feedback inhibition, and optimizing the supply of precursors.

Tryptophan production in *Escherichia coli* involves the shikimate pathway, which is responsible for the biosynthesis of aromatic amino acids. This pathway begins with the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate to form 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), catalyzed by DAHP synthase. Subsequent steps involve the formation of shikimate, chorismate, and finally tryptophan through the actions of enzymes such as anthranilate synthase and tryptophan synthase. Enhancing tryptophan production typically involves overexpressing genes encoding these enzymes, knocking out competing pathways, and increasing the availability of precursor molecules.

Methionine is produced via the aspartate family pathway, which converts aspartate to homoserine, then to O-succinylhomoserine, and ultimately to methionine through several enzymatic steps. Key enzymes in this pathway include aspartokinase, homoserine dehydrogenase, and cystathionine gamma-synthase. Genetic engineering strategies to boost methionine production involve overexpressing these enzymes, removing regulatory constraints, and ensuring a robust supply of precursors.

Genetic Engineering Strategies

Advancements in genetic engineering have significantly enhanced the microbial production of vitamins and amino acids. These strategies involve precise modifications to the genetic makeup of microorganisms to optimize metabolic pathways, increase production yields, and improve process efficiency. Key genetic engineering strategies include pathway optimization, overexpression of biosynthetic genes, deletion of regulatory genes, and introduction of heterologous pathways.

Metabolic pathway optimization is crucial for enhancing the production of target compounds. This strategy involves identifying and modifying rate-limiting steps in the biosynthetic pathways. Techniques such as metabolic flux analysis and synthetic biology tools are employed to map the metabolic network and pinpoint bottlenecks. By overexpressing or mutating specific enzymes that catalyze these rate-limiting steps, the metabolic flux can be redirected towards the desired product. For example, in the production of lysine in Corynebacterium glutamicum, the overexpression of key enzymes such as aspartokinase and dihydrodipicolinate synthase can significantly increase lysine yields by enhancing the flow through the diaminopimelate pathway.

One of the most straightforward and effective strategies in genetic engineering is the overexpression of genes encoding key enzymes in the biosynthetic pathways. This is achieved by introducing strong promoters that drive high levels of gene expression. In the case of riboflavin production, overexpressing genes such as ribA (Encoding GTP cyclohydrolase II) and ribB (Encoding 3,4-dihydroxy-2-butanone-4-phosphate synthase) in Bacillus subtilis can lead to significantly increased riboflavin output. This strategy ensures that the rate of riboflavin synthesis is maximized by providing an ample supply of the enzymes that catalyze the key steps in the pathway.

Feedback inhibition is a common regulatory mechanism in metabolic pathways, where the end product inhibits the activity of enzymes involved in its own biosynthesis. Deleting genes that encode these regulatory proteins can prevent feedback inhibition and enhance production yields. For example, in the microbial production of lysine, deleting the gene encoding the lysine repressor protein (lysR) in Corynebacterium glutamicum removes the feedback inhibition on the lysine biosynthetic pathway. This allows for uninterrupted flow through the pathway, leading to higher lysine accumulation. Similarly, in the production of tryptophan in *Escherichia coli*, the deletion of the tryptophan repressor gene (trpR) can result in increased tryptophan synthesis.

Introducing biosynthetic pathways from other organisms into a host microorganism can enable the production of compounds that the host does not naturally synthesize. This strategy involves cloning and expressing genes from the donor organism into the host. For instance, the microbial production of vitamin C (Ascorbic acid) can be achieved by introducing the plant-based biosynthetic pathway into yeast or bacteria. This involves cloning genes such as GDP-mannose pyrophosphorylase and GDP-mannose-3, 5-epimerase from plants into a microbial host. These enzymes catalyze the conversion of glucose to ascorbic acid, allowing the microorganism to produce vitamin C efficiently.

Synthetic biology tools and CRISPR-Cas9 genome editing have revolutionized genetic engineering by enabling precise and efficient modifications. CRISPR-Cas9 allows for targeted gene editing, where specific genes can be knocked out, inserted, or modified with high precision. This technology can be used to engineer microbial strains with enhanced production capabilities. For example, using CRISPR-Cas9 to knock out competing pathways or to insert genes that enhance cofactor regeneration can significantly boost the production of desired metabolites. In synthetic biology, modular and standardized genetic parts (such as promoters, ribosome binding sites, and terminators) are assembled to construct optimized metabolic pathways. This approach allows for the systematic tuning of gene expression and pathway fluxes, leading to improved production efficiency.

ALE is a complementary strategy that involves subjecting microorganisms to selective pressure over multiple generations to evolve strains with desirable traits. This approach can be used to develop strains with enhanced tolerance to production conditions or improved metabolic efficiency. For example, evolving a microbial strain under high concentrations of a target amino acid can select for mutants with increased production capacity. The evolved strains can then be analyzed to identify beneficial genetic changes, which can be further optimized using genetic engineering.

Bioprocess Optimization Techniques

Bioprocess optimization is a critical aspect of enhancing microbial production of vitamins and amino acids. It involves fine-tuning various parameters and processes to maximize yield, efficiency, and overall productivity. This encompasses the optimization of fermentation conditions, nutrient composition, and the use of advanced bioreactor systems.

Fermentation conditions play a pivotal role in microbial production. Controlling parameters such as pH, temperature, and aeration is essential for maximizing microbial growth and product formation. For example, the production of vitamin C by Gluconobacter oxydans can be significantly enhanced by maintaining an optimal pH and aeration rate. Precise control of these parameters ensures that the microorganism operates in its optimal growth range, leading to higher product yields. Temperature optimization is equally important as it affects the metabolic rate of the microorganisms. For instance, the production of lysine in Corynebacterium glutamicum is optimized at a specific temperature range, where the enzymatic activities involved in lysine biosynthesis are at their peak.

Nutrient optimization is another crucial factor in bioprocess optimization. The composition of the growth medium has a profound impact on microbial metabolism and product yield. Providing the necessary precursors and cofactors can enhance the biosynthetic pathways. For example, in the production of riboflavin by Bacillus subtilis, the addition of suitable carbon and nitrogen sources can boost riboflavin synthesis. Similarly, the production of tryptophan in *Escherichia coli* can be enhanced by optimizing the supply of key nutrients such as glucose and ammonium sulfate. In addition, the use of fed-batch fermentation, where nutrients are added gradually, can prevent nutrient depletion and maintain microbial growth in the exponential phase, leading to higher product yields. The use of bioreactors allows for precise control of fermentation conditions and scaling up the production process. Advanced bioreactor designs, such as fed-batch and continuous fermentation systems, can further enhance production efficiency. Fed-batch fermentation involves the intermittent addition of nutrients to the bioreactor, preventing nutrient depletion and maintaining optimal growth conditions. This approach is particularly effective for the production of amino acids such as lysine and methionine, where maintaining a steady supply of precursors is crucial for high yields. Continuous fermentation systems, on the other hand, involve the constant addition of fresh medium and removal of spent medium, allowing for sustained microbial growth and product formation. This method is advantageous for producing vitamins and amino acids that are required in large quantities. Aeration and oxygen transfer are also critical parameters in bioprocess optimization. Many microbial production processes are aerobic, requiring efficient oxygen transfer to support high metabolic activity. The design of the bioreactor, including the type and placement of spargers and agitators, affects oxygen transfer rates. For instance, in the production of glutamate by Corynebacterium glutamicum, optimizing the aeration rate and using an efficient agitation system can significantly enhance glutamate yields. Oxygen transfer can be further improved by using oxygen-enriched air or pure oxygen in the fermentation process. Metabolic flux analysis (MFA) is a powerful tool for bioprocess optimization. MFA involves the quantification of metabolic fluxes within the microbial cell, providing insights into the distribution of metabolic pathways and identifying bottlenecks. By using isotopic labeling and advanced analytical techniques, researchers can map the flow of metabolites through different pathways. This information can be used to modify the fermentation process, such as adjusting nutrient supply or optimizing aeration, to redirect metabolic fluxes towards the desired product. For example, in the production of tryptophan, MFA can identify competing pathways that divert precursors away from tryptophan synthesis, allowing for targeted genetic modifications and process adjustments to enhance tryptophan yield. Process monitoring and control are essential for maintaining optimal conditions throughout the fermentation process. Advanced sensors and control systems can continuously monitor key parameters such as pH, temperature, dissolved oxygen, and nutrient levels. Automated control systems can adjust these parameters in real-time to maintain optimal conditions, ensuring consistent product quality and yield. For example, in the production of vitamin B12 by Propionibacterium freudenreichii, automated control of pH and dissolved oxygen levels can significantly improve vitamin B12 yields. Downstream processing is another important aspect of bioprocess optimization. Efficient separation and purification of the desired product from the fermentation broth are crucial for achieving high product purity and yield. Techniques such as centrifugation, filtration, and chromatography are commonly used for downstream processing. Optimizing these processes can reduce production costs and improve overall efficiency. For instance, in the production of riboflavin, optimizing the extraction and purification process can significantly increase the final product yield and reduce impurities.

Conclusion

Microbial production of vitamins and amino acids represents a sustainable and efficient alternative to traditional chemical synthesis methods. Through the application of advanced genetic engineering strategies, metabolic pathways in microorganisms can be optimized to enhance the production of these essential nutrients. Additionally, bioprocess optimization techniques, including the precise control of fermentation conditions, nutrient composition, and the use of sophisticated bioreactor systems, have significantly improved production yields and process efficiency.

The integration of pathway optimization, gene overexpression, regulatory gene deletion, and the introduction of heterologous pathways has led to the development of microbial strains with superior production capabilities. Tools such as CRISPR-Cas9 and synthetic biology further enhance our ability to engineer microorganisms for high-level production of vitamins and amino acids. Moreover, the use of metabolic flux analysis and advanced process monitoring ensures that bioprocess conditions are maintained at optimal levels, maximizing productivity and consistency.

As the demand for vitamins and amino acids continues to grow in the nutraceutical and pharmaceutical industries, microbial production offers a promising solution. It provides a renewable and environmentally friendly approach to producing these critical nutrients, reducing reliance on chemical synthesis and minimizing environmental impact. Future advancements in genetic engineering, bioprocess technology, and systems biology will likely further enhance the efficiency and scalability of microbial production systems, supporting the development of more sustainable and cost-effective production methods.

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