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Research Paper

A Review on Microbial Production of Ergothioneine: Current Advances and Future Prospects in Lactobacillus-Based Fermentation

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Abstract

Ergothioneine (ERG) is a potent antioxidant with cytoprotective and anti-inflammatory properties, offering therapeutic potential for oxidative stress-related diseases like cardiovascular and neurodegenerative disorders. Despite its documented health benefits, the full scope of ERG's biological functions remains underexplored. This review focuses on microbial production of ERG, particularly through lactic acid bacteria (LAB) such as Lactobacillus reuteri, which synthesizes ERG via ergothioneine synthase (EgtA). We discuss microbial biosynthesis pathways, strain selection, and metabolic engineering strategies to enhance ERG production. Additionally, the review examines the role of fermentation conditions, including temperature, pH, and nutrient media, in optimizing ERG yields. ERG's applications in functional foods, nutraceuticals, and skincare are highlighted, emphasizing its sustainable and cost-effective production through LAB fermentation. Challenges in scaling up production, optimizing fermentation, and downstream processing are also addressed. Future research aims to improve biotechnological methods, bioreactor design, and metabolic engineering to enhance production efficiency and commercial viability. ERG shows great promise as a bioactive compound for health promotion, and further advancements in microbial production could offer innovative solutions for oxidative stress-related diseases.

Keywords: *Ergothioneine, antioxidants, lactic acid bacteria, fermentation, metabolic engineering, sustainable production.*

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1. INTRODUCTION

Ergothioneine (ERG) is a naturally occurring compound with growing evidence supporting its role in human health, particularly for its cytoprotective properties in maintaining cellular integrity and combating oxidative stress.

While its biological activities are well documented, its full range of functions remains largely unexplored. Ergothioneine levels are often lower in individuals with chronic inflammatory diseases, spurring interest in its potential as a therapeutic nutraceutical and antioxidant. However, its exact mechanisms of action and broader medical applications are still under investigation. As of now, ergothioneine is not classified alongside established biologically active compounds like vitamins or hormones due to limited understanding of its role in human physiology **(Donald B. Melville et al., 1959)**. A key area of research is the relationship between ergothioneine and fermentation processes, especially with lactic acid bacteria (LAB), such as Lactobacillus species.

LABs are critical in fermenting food products, enhancing both their preservation and nutritional value. Recent studies show that Lactobacillus-fermented mushroom products, enriched with bioactive compounds, can inhibit obesity-related metabolic enzymes and reduce inflammation. These fermented products hold promise as functional probiotic supplements or food items **(Jun Hui Choi et al., 2021)**. Lactic acid bacteria are also central to functional foods, offering health benefits ranging from improved gut health to immune modulation. With the growing demand for probiotics, understanding how LABs withstand stress conditions, like glucose limitation, is vital for optimizing industrial fermentation. One study using Lactobacillus casei Zhang, which evolved under glucose restriction for 4,000 generations, provided valuable insights into the bacterium's metabolic adaptations, helping to optimize LAB strains for food production **(Lin Pan et al., 2019)**.

Beyond bacteria, liquid fermented products from edible and medicinal fungi are emerging as another promising area for enhancing food products. Fungi, known for their therapeutic properties, can be fermented using advanced technologies to amplify their bioactive compounds, offering benefits like immune support, anti-inflammatory effects, and antioxidants. However, further optimization of fermentation processes and safety measures is needed, along with studies into the synergistic effects of combining fermented fungi with other food ingredients to boost their nutritional value **(Meng Qiu Yan et al., 2023)**. Additionally, research on low-molecular-weight thiols in bacteria has deepened our understanding of how these microorganisms protect themselves from environmental stress. Thiols like glutathione, mycothiol, and bacillithiol play antioxidant roles, with ergothioneine drawing particular attention for its protective functions.

Although much remains to be learned about its biosynthesis and distribution in different bacterial species, studies on thiol systems, including ergothioneine in Actinobacteria and bacillithiol in Firmicutes, highlight their importance in bacterial stress response **(Robert C. Fahey, 2013)**. The current research into ergothioneine, lactic acid bacteria, and liquid fermented products is paving the way for the development of functional foods and nutraceuticals. Ergothioneine shows great therapeutic potential, though its biological functions are still being explored. Meanwhile, LABs and fermented fungi offer promising avenues to enhance food products with health benefits, particularly in addressing metabolic and inflammatory diseases. As fermentation technologies advance and our understanding of bacterial protective systems grows, the future of functional foods looks promising, with new opportunities to improve human health through innovative dietary solutions.

2. MICROBIAL PRODUCTION OF ERGOTHIONEINE

Ergothioneine is a naturally occurring antioxidant with potent cytoprotective properties, produced by various microorganisms, including fungi, actinobacteria, and bacteria. Among fungi, Aspergillus and Penicillium species are prominent producers, using complex biosynthetic machinery to convert precursor compounds into ergothioneine. Certain actinobacteria, such as Mycobacterium and Streptomyces, also synthesize ergothioneine, likely as a defense against oxidative stress and other environmental challenges. In bacteria, Lactobacillus strains, particularly Lactobacillus reuteri, produce ergothioneine through the action of the enzyme ergothioneine synthase (EgtA), which catalyzes the conversion of histidine and sulfur-containing precursors into ergothioneine. The presence of EgtA enables L. reuteri and other Lactobacillus species to synthesize ergothioneine, which serves as a protective antioxidant, enhancing bacterial resilience to oxidative stress. The biosynthetic pathway starts with histidine, an amino acid, which is modified by EgtA and sulfur compounds to form ergothioneine **(Lind et al., 2005)**. This pathway is crucial for understanding microbial antioxidant defense mechanisms and for optimizing ergothioneine production in these organisms, with potential applications in industrial and therapeutic contexts.

3. LACTOBACILLUS SPECIES FOR ERGOTHIONEINE PRODUCTION

❖ Lactobacillus spp. Overview

Lactobacillus species are a diverse group of lactic acid bacteria (LAB) widely recognized for their probiotic properties, which support gastrointestinal health, immune modulation, and the production of bioactive metabolites. These bacteria are commonly found in fermented foods like yogurt, kefir, and sauerkraut, where they primarily produce lactic acid as a metabolic byproduct. However, certain Lactobacillus species, including Lactobacillus reuteri, are also capable of producing other valuable metabolites, such as the antioxidant ergothioneine. This thiol compound, known for its potent cytoprotective and antioxidant properties, has attracted attention for its potential health benefits, offering a unique probiotic advantage beyond the typical roles of fermentation and digestion **(Lind et al., 2005; Basha Tang et al., 2022)**.

➢ Strain Selection

Among Lactobacillus strains, Lactobacillus reuteri and Lactobacillus plantarum are the most studied for their ability to produce ergothioneine. L. reuteri is particularly noted for its probiotic potential and its capacity to synthesize ergothioneine via the enzyme ergothioneine synthase (EgtA), which efficiently converts histidine and sulfur-containing precursors into ergothioneine, making it a strong candidate for antioxidant production **(Lind et al., 2005)**. Other strains, such as L. plantarum, also produce ergothioneine, though at varying levels, and are under investigation for their potential in ergothioneine synthesis **(Lind et al., 2005)**. The selection of efficient strains for ergothioneine production depends on their metabolic capabilities, environmental resilience, and overall yield.

➢ Genetic and Metabolic Pathways

In Lactobacillus species, especially L. reuteri, ergothioneine biosynthesis is catalyzed by EgtA, which plays a crucial role in converting histidine and sulfur precursors into ergothioneine. Research by **Chiara et al. (2017)** identified the genetic loci responsible for ergothioneine production in L. reuteri and highlighted key genes involved in the EgtA-mediated biosynthesis. These findings provide insights into the metabolic network supporting ergothioneine production and suggest that manipulating gene expression could enhance yields. Metabolic engineering shows great promise in optimizing ergothioneine biosynthesis in Lactobacillus strains. Modifying specific pathways—such as increasing the availability of sulfur precursors or enhancing EgtA expression—could significantly boost ergothioneine production. This approach could facilitate the development of industrial-scale fermentation processes for producing ergothioneine-rich Lactobacillus strains, with potential applications in functional foods, nutraceuticals, and therapeutics. In summary, Lactobacillus species, particularly L. reuteri and L. plantarum, are promising candidates for microbial ergothioneine production. Their probiotic properties, coupled with the ability to synthesize this valuable antioxidant, make them attractive for both food and health applications. Ongoing research into the genetic and metabolic pathways, along with efforts in metabolic engineering, could lead to more efficient and sustainable production of this bioactive compound for industrial use.

4. FERMENTATION PROCESS OPTIMIZATION FOR ERGOTHIONEINE PRODUCTION

The production of ergothioneine through microbial fermentation is highly influenced by several fermentation parameters that directly affect microbial growth and the synthesis of this bioactive compound. Key factors such as temperature, pH, nutrient medium, aeration, and inoculum size play critical roles in optimizing ergothioneine production. Temperature significantly impacts microbial metabolism, with Lactobacillus strains like L. reuteri performing optimally between 30°C and 37°C. Deviations from this range can impair microbial activity and reduce ergothioneine yield. The pH of the fermentation medium is also crucial, as Lactobacillus strains thrive in mildly acidic environments (pH 5.5–6.5), which support the stability of enzymes like ergothioneine synthase (EgtA). The nutrient medium composition, particularly the carbon and nitrogen sources, also affects ergothioneine production. Carbon sources like glucose and sucrose provide energy, while nitrogen sources such as ammonium salts are essential for protein synthesis and metabolism. Optimizing these nutrients has been shown to significantly enhance ergothioneine yields.

Aeration and oxygen levels are another critical aspect of the fermentation process. While Lactobacillus strains typically grow in anaerobic or microaerophilic conditions, limited oxygen availability can stimulate ergothioneine production and other antioxidants. Agitation or aeration, such as shaking or stirring, improves oxygen transfer, ensuring optimal growth conditions. The initial inoculum size also impacts fermentation efficiency. A higher inoculum accelerates the process but can lead to overcrowding and nutrient depletion if excessive, reducing ergothioneine yields. To further enhance ergothioneine production, several strategies have been explored. Genetic engineering of Lactobacillus strains offers a promising approach. By modifying genes involved in ergothioneine biosynthesis, such as egtA, researchers can improve production efficiency.

Additionally, metabolic engineering can enhance sulfur precursor uptake and optimize sulfur metabolism enzymes, crucial for ergothioneine synthesis. Medium supplementation, especially with sulfur-containing amino acids like cysteine and methionine, can also boost ergothioneine yields. Bioreactors are valuable for providing controlled environments where fermentation parameters—such as pH, temperature, aeration, and nutrient supply—can be precisely optimized. Bioreactor systems, especially continuous or fed-batch setups, ensure stable and efficient large-scale production, making it possible to meet the demands of industrial and therapeutic applications. In summary, optimizing fermentation conditions through methods like genetic engineering, medium supplementation, and bioreactor utilization can significantly enhance ergothioneine production. These strategies offer great potential for large-scale, cost-effective production, supporting the use of ergothioneine in functional foods, nutraceuticals, and health-promoting applications (Rios-Covian et al., 2016).

5. BIOTECHNOLOGICAL APPLICATIONS AND POTENTIAL OF ERGOTHIONEINE

❖ Applications in Health

Ergothioneine is a potent antioxidant with significant potential for various health-related applications. Its strong antioxidative properties make it an attractive candidate for inclusion in dietary supplements and nutraceuticals aimed at mitigating oxidative stress, which is linked to chronic diseases such as cardiovascular conditions, neurodegenerative disorders (e.g., Alzheimer’s and Parkinson’s), and inflammatory diseases. By neutralizing reactive oxygen species (ROS), ergothioneine helps protect cells and tissues from oxidative damage, supporting overall cellular health. In addition to its internal health benefits, ergothioneine’s cytoprotective qualities extend to skin care, where it is being explored for reducing UV-induced skin damage, preventing premature aging, and enhancing skin rejuvenation. As a result, it is increasingly used in anti-aging creams and dermatological products. Its ability to modulate immune responses and reduce inflammation further enhances its appeal as a potential therapeutic agent for a variety of medical conditions.

❖ Sustainable Production

Traditionally, ergothioneine has been extracted from fungi or synthesized chemically, both of which can be resource-intensive and environmentally damaging. In contrast, Lactobacillus-based fermentation provides a more sustainable and eco-friendlier alternative. This biotechnological approach uses renewable resources, such as sugars and amino acids, and relies on microbial cultures to produce ergothioneine under controlled conditions. Fermentation is more energy-efficient and generates fewer byproducts, making it a greener method compared to conventional chemical synthesis. Furthermore, the fermentation process can be optimized for scalability and efficiency, enabling large-scale production. By utilizing Lactobacillus strains, particularly Lactobacillus reuteri, ergothioneine can be produced more sustainably and cost-effectively. This method aligns with the growing demand for natural, environmentally conscious manufacturing processes.

❖ **Commercial Viability**

Despite the advantages of Lactobacillus-based fermentation, several challenges remain when scaling up ergothioneine production for industrial applications. One key hurdle is optimizing the fermentation process to achieve consistently high yields. Factors such as nutrient composition, temperature, pH, and oxygen levels must be precisely controlled to maximize ergothioneine output. Additionally, the cost of raw materials, particularly carbon and nitrogen sources, can be significant, and fluctuations in raw material prices could affect overall production costs. Another challenge is the downstream processing required to isolate and purify ergothioneine from the fermentation broth, which adds complexity and expense to the process. Furthermore, achieving commercial viability will necessitate further advancements in bioreactor design, metabolic engineering, and medium optimization to enhance both yield and cost-effectiveness. While large-scale, cost-efficient production is feasible, addressing these challenges is crucial for the commercial success of Lactobacillus-based ergothioneine production. As biotechnological innovations continue to refine these processes, ergothioneine's commercial potential in health, skincare, and nutraceuticals is expected to grow, providing a more sustainable and economically viable alternative to traditional production methods **(Calo et al., 2020)**.

6. CHALLENGES AND FUTURE DIRECTIONS

While Lactobacillus strains, particularly Lactobacillus reuteri, hold promise for ergothioneine production, they face notable limitations when compared to other microorganisms such as fungi (Aspergillus and Penicillium) and actinobacteria, which naturally produce higher quantities of the antioxidant. The primary challenge for Lactobacillus strains is the relatively low expression of ergothioneine synthase (EgtA), the enzyme responsible for ergothioneine biosynthesis. Additionally, the biosynthetic pathway in Lactobacillus is less efficient than in other producers, resulting in lower yields. Further complicating matters, Lactobacillus species have slower growth rates and metabolic limitations, which hinder the production of high ergothioneine concentrations under standard fermentation conditions. Optimizing fermentation for Lactobacillus involves addressing complex technical challenges, such as controlling nutrient media composition, pH, temperature, oxygen supply, and agitation rates, all of which must be precisely managed to maximize yield. Achieving consistent, large-scale production remains a significant hurdle, as fluctuations in these factors can negatively affect microbial metabolism and overall productivity **(Wu et al., 2017)**.

7. CONCLUSION

In conclusion, ergothioneine emerges as a promising bioactive compound with potent antioxidant and cytoprotective properties, offering significant potential for therapeutic applications in health, skincare, and nutraceuticals. While its full range of biological functions remains under investigation, its protective effects against oxidative stress and inflammatory diseases position it as an important candidate for inclusion in functional food products and medical treatments. Microbial production of ergothioneine, particularly through lactic acid bacteria (LAB) like Lactobacillus reuteri, presents an exciting alternative to traditional chemical or fungal extraction methods, offering a more sustainable and cost-effective pathway for large-scale production. Ongoing research into optimizing fermentation conditions, metabolic engineering, and the genetic pathways involved in ergothioneine synthesis in Lactobacillus strains holds promise for enhancing production yields and improving efficiency. However, challenges remain in scaling up the fermentation process, including optimizing nutrient media, fermentation parameters, and downstream processing techniques. Overcoming these barriers will be crucial for realizing the commercial potential of ergothioneine and ensuring its accessibility for industrial applications.

The future of ergothioneine production is likely to benefit from continued advancements in biotechnology, including bioreactor optimization and emerging fermentation technologies. As our understanding of its biological functions and biosynthesis mechanisms deepens, and as production methods become more efficient, ergothioneine may become a widely utilized compound in health-promoting products, paving the way for more sustainable and effective solutions in the treatment and prevention of various oxidative stress-related diseases. Further research in this field will be pivotal in unlocking the full potential of ergothioneine, positioning it as a key player in the future of functional foods and therapeutic interventions.

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