



## Development of Novel Fermented Tea Products through Microbial Community Engineering

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**Abstract** The field of fermented tea products is witnessing a transformative phase with the incorporation of microbial community engineering techniques. This review paper explores the development of novel fermented tea products by leveraging advances in microbial ecology and meta-omics. By examining various case studies, including Pu-erh, Liu-bao, Fu-brick, and Miang teas, this paper highlights the significant impact of microbial manipulations on tea fermentation processes. Integrated meta-omics approaches have uncovered the complex interactions within microbial communities and their direct roles in enhancing the flavor, aroma, and health-promoting properties of fermented teas. Specific attention is given to the role of engineered microbes such as *Aspergillus niger* and the utilization of microbial enzymes for targeted flavor profile enhancements. Furthermore, this review discusses the technical, scale-up, and regulatory challenges faced in the commercialization of these innovations. The potential market opportunities for these engineered products are also assessed, reflecting consumer trends towards health-centric and gourmet beverage options. This paper aims to provide a comprehensive overview of current methodologies and future directions in the development of fermented tea products through microbial community engineering, paving the way for new possibilities in the beverage industry.

**Keywords** Fermented tea; Microbial community engineering; Meta-omics; Flavor enhancement; Pu-erh tea; Liu-bao tea; Fu-brick tea; Miang tea; Health benefits; *Aspergillus niger*; Microbial enzymes

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Fermented tea products, such as Kombucha, Pu-erh, Liubao, and Miang, have been consumed for centuries, particularly in Asian cultures. These traditional beverages are made from the leaves of *Camellia sinensis* and owe their unique flavors, textures, and health benefits to the complex microbial communities that drive the fermentation process (Chakravorty et al., 2016; Zhao et al., 2019; Unban et al., 2020). The microbial consortia typically consist of a variety of bacteria and yeasts, which interact in a dynamic ecosystem to produce a range of organic acids, enzymes, and bioactive compounds (Chakravorty et al., 2016; Zhao et al., 2019; Unban et al., 2020; Long et al., 2023).

The importance of microbial communities in the fermentation process cannot be overstated. They are responsible for the transformation of tea polyphenols into a spectrum of new compounds, some of which are associated with the antioxidant, antimicrobial, and potentially probiotic properties of the fermented tea (Chakravorty et al., 2016; Zhao et al., 2019). For instance, during the fermentation of Miang, a traditional fermented tea leaf from northern Thailand, lactic acid bacteria (LAB) and yeasts such as *Candida* and *Pichia* play a pivotal role in the development of the product's characteristics (Unban et al., 2020). Similarly, in Kombucha tea fermentation, the microbial community, including genera like *Komagataibacter* and *Candida*, is crucial for the biochemical dynamics that confer the beverage's beneficial properties (Chakravorty et al., 2016).

Given the critical role of these microorganisms, there is a growing rationale for engineering microbial communities to develop novel tea products. By manipulating the microbial consortia, it is possible to enhance desirable features such as flavor profiles, nutritional value, and health benefits, while also ensuring product safety and consistency (Chakravorty et al., 2016; Zhao et al., 2019; Unban et al., 2020). For example, the application of

enzymatic fermented soybean in tea plantations has been shown to regulate microbial communities in the soil, positively affecting the lipid metabolites in tea new shoots (Zhang et al., 2022). This suggests that targeted interventions in microbial communities can have far-reaching effects on the tea itself and its surrounding ecosystem.

The objectives of this research are to explore the potential of microbial community engineering in the development of novel fermented tea products, to understand the interactions between different microbial species during fermentation, and to identify key enzymes and metabolites involved in the process. We expect that this research will lead to the creation of new tea products with enhanced flavors, health benefits, and commercial value, while also contributing to the sustainability of tea agriculture through improved soil health and reduced reliance on chemical fertilizers (Zhao et al., 2015; Zhao et al., 2019; Zhang et al., 2022; Long et al., 2023).

## 1 Fundamentals of Tea Fermentation

### 1.1 Basic principles of tea fermentation

Tea fermentation is a complex biochemical process that involves the microbial transformation of tea leaf components into a variety of new compounds that contribute to the unique flavors, aromas, and colors of fermented tea products. The process is largely anaerobic and can be either spontaneous or controlled, depending on the desired outcome of the fermentation. During fermentation, microorganisms such as bacteria, yeasts, and fungi metabolize the tea's polyphenols, amino acids, and carbohydrates, leading to the production of a range of bioactive compounds (Wang et al., 2015; Li et al., 2018b; Zhao et al., 2019; Hu et al., 2022).

### 1.2 Key microbial players and their roles in tea fermentation

The microbial community in tea fermentation is diverse and includes a variety of fungi and bacteria that play crucial roles in the development of the tea's characteristics. *Aspergillus*, *Candida*, *Lactobacillus*, and *Komagataibacter* are some of the key genera identified in different stages of tea fermentation. *Aspergillus* species, for instance, are significant in the early stages of fermentation, contributing to the formation of flavor compounds such as theabrownin and methoxy-phenolic compounds (Chakravorty et al., 2016; Li et al., 2018a; Zou et al., 2022). Yeasts like *Candida* and bacteria such as *Lactobacillus* and *Komagataibacter* are also involved in the fermentation process, influencing the biochemical properties and beneficial health effects of the tea (Chen, 2012; Chakravorty et al., 2016).

### 1.3 Factors influencing microbial activity and tea fermentation dynamics

Several factors influence the activity of the microbial community and the dynamics of tea fermentation. These include the temperature and pH of the fermentation environment, the availability of nutrients, and the moisture content of the tea leaves. For example, the temperature of the fermentation pile can affect the growth and activity of the microbial community, as seen in the fermentation of Puer tea, where the temperature is maintained around 50 °C for optimal microbial activity (Zou et al., 2022). The water content and pH also play a role in creating favorable conditions for the propagation of specific fungi and bacteria (Zou et al., 2022). Additionally, the presence of enzymes such as polyphenol oxidase, cellulase, and pectinase, which are produced by the microbial community, can significantly alter the chemical composition of the tea, affecting its flavor and health properties (Li et al., 2018b; Zhu et al., 2019).

In conclusion, the fermentation of tea is a dynamic process governed by the interplay of microbial communities and environmental factors. Understanding these fundamentals is essential for the development of novel fermented tea products with specific nutritional and health benefits.

## 2 Microbial Community Engineering: Concepts and Techniques

### 2.1 Definition of microbial community engineering

Microbial community engineering is an emerging field that focuses on the intentional design and manipulation of microbial communities to achieve specific outcomes or enhance certain functions. This discipline recognizes the complexity of interactions within microbial ecosystems, including species-species and species-environment

interactions, as well as the feedbacks between ecological and evolutionary dynamics (eco-evo feedbacks) (Zerfaß et al., 2018). The goal is to create stable and efficient communities that can perform desired tasks more effectively than individual species or unengineered communities.

## 2.2 Overview of techniques used in microbial engineering

### 2.2.1 Genetic engineering of microbes

Genetic engineering of microbes involves modifying the genetic material of microorganisms to alter their capabilities or behaviors. This can include adding, removing, or modifying specific genes to confer new metabolic pathways, resistance to toxins, or the ability to produce valuable compounds. Advances in molecular techniques, such as CRISPR-Cas systems, have greatly enhanced the precision and efficiency of genetic modifications in microbial engineering (Sheth et al., 2016).

### 2.2.2 Synthetic microbial communities (SynComs)

Synthetic microbial communities, or SynComs, are designed by combining selected microorganisms to form a community with desired characteristics. This approach can involve the artificial selection of naturally occurring microbes, reduction from existing communities, or combinatorial evaluation of potential microbial combinations. Computational methods are increasingly used to optimize the design of SynComs for specific applications<sup>5</sup>.

### 2.2.3 Adaptive laboratory evolution

Adaptive laboratory evolution (ALE) is a technique where microbial populations are exposed to controlled environmental conditions over extended periods, allowing for the natural selection of advantageous traits. This method can lead to the development of strains with improved performance characteristics, such as increased tolerance to stress or enhanced metabolic efficiency (Zerfaß et al., 2018).

### 2.2.4 Use of prebiotics and probiotics to modulate microbial communities

The use of prebiotics and probiotics represents a strategy to modulate microbial communities by providing nutrients that selectively promote the growth of beneficial microbes (prebiotics) or by directly adding beneficial microorganisms to the community (probiotics). This approach can influence community-level properties and has applications in human health, agriculture, and biotechnology (Sheth et al., 2016).

In summary, microbial community engineering is a multifaceted field that employs a variety of techniques to design and manipulate microbial ecosystems. These techniques range from genetic modifications of individual species to the construction of complex communities with specific functions. The ultimate aim is to harness the collective capabilities of microbes to address challenges in various domains, including environmental management, industrial bioprocessing, and health (Sheth et al., 2016; Zerfaß et al., 2018; Eng and Borenstein, 2019).

## 3 Recent Advances in Microbial Community Engineering for Tea Fermentation

Recent advancements in microbial community engineering have significantly impacted the fermentation process of tea, leading to the development of novel fermented tea products with enhanced flavor, aroma, and health benefits. This section of the review paper will explore various case studies, genetic modifications, applications of synthetic communities (SynComs), and the overall impact of engineered microbial communities on the fermentation efficiency and product consistency of tea.

### 3.1 Case studies of engineered microbial communities in tea fermentation

Several studies have demonstrated the role of microbial communities in the fermentation of traditional teas. For instance, the non-filamentous fungi growth-based fermentation process of Miang, a traditional fermented tea of North Thailand, showed that lactic acid bacteria (LAB), yeast, and *Bacillus* were the main microbial populations throughout the fermentation period (Unban et al., 2020). Similarly, the microbial community analysis of Sichuan South-road Dark Tea (SSDT) revealed that *Aspergillus niger* M10, isolated from SSDT, was excellent at enhancing organoleptic qualities such as soluble sugar, amino acids, and thearubigins contents (Zou et al., 2022).

These case studies highlight the potential of manipulating microbial communities to improve the quality of fermented tea products.

### **3.2 Outcomes of genetic modifications in key microbial species**

Genetic modifications in key microbial species have been shown to influence the fermentation process and the quality of the final tea product. For example, *Aspergillus niger* M10 was found to produce glycoside hydrolases, which are enzymes that contribute to the sweet and mellow taste of SSDT during fermentation (Zou et al., 2022). In another study, the integrated metagenomics/metaproteomics approach identified *Aspergillus* as the dominant fungus and major host of identified proteins, which are involved in the degradation of the plant cell wall and oxidation of catechins during the solid-state fermentation of Pu-erh tea (Zhao et al., 2015).

### **3.3 Applications of SynComs in enhancing flavor, aroma, and health benefits**

The application of synthetic microbial communities (SynComs) has been explored to enhance the flavor, aroma, and health benefits of fermented tea products. For instance, the microbial diversity and interaction specificity in Kombucha tea fermentations were studied, revealing that manipulation of key taxa in kombucha microbiomes could shape the fermentation qualities and production of biofilms (Landis et al., 2022). This suggests that SynComs can be designed to optimize the fermentation process for desired outcomes.

### **3.4 Impact of engineered communities on fermentation efficiency and product consistency**

Engineered microbial communities have been shown to impact the fermentation efficiency and product consistency positively. The study on the microbial diversity and interaction specificity in Kombucha tea fermentations indicated that specific microbial interactions within kombucha biofilms could lead to more consistent fermentation qualities (Landis et al., 2022). Additionally, the microbial and biochemical dynamics during the fermentation of Kombucha tea were explored, suggesting that the microbial community structure and dynamics play a crucial role in the biochemistry of the fermentation process (Chakravorty et al., 2016).

In conclusion, recent advances in microbial community engineering offer promising strategies for optimizing the fermentation process of tea. By understanding and manipulating microbial communities, it is possible to enhance the quality and consistency of fermented tea products, catering to the evolving preferences of consumers worldwide.

## **4 Challenges and Opportunities**

### **4.1 Technical challenges in microbial engineering for tea products**

Microbial engineering of tea products presents several technical challenges that need to be addressed to harness its full potential. One of the primary issues is the complexity of tea's microbial ecosystem. Engineering these communities requires a deep understanding of microbial interactions and their impact on tea flavor, health benefits, and stability. Moreover, maintaining the consistency of the modified microbial traits during the fermentation process is challenging due to the dynamic nature of microbial growth and interaction, which can be influenced by slight variations in fermentation conditions.

### **4.2 Scale-up issues from laboratory to commercial production**

Scaling up microbial engineering from the laboratory to commercial production poses significant hurdles. Processes developed in the lab are often conducted on a small scale and under ideal conditions, which do not necessarily translate to larger production systems. Scaling up involves optimizing the growth media, fermentation parameters, and environmental controls to maintain the desired microbial activity and product quality. Additionally, issues such as the cost of production, efficiency at larger scales, and consistency between batches must be carefully managed to ensure commercial viability.

### **4.3 Regulatory and safety considerations for genetically modified organisms (GMOs) in food products**

The use of genetically modified organisms (GMOs) in food products, including fermented tea, brings about stringent regulatory and safety considerations. Regulatory frameworks vary significantly by country, affecting everything from GMO development to market entry. The primary concerns include ensuring that GMOs do not

introduce allergens or toxins into the food supply and that they do not adversely affect the environment. Public perception and acceptance of GMO-containing products also play a crucial role in the regulatory landscape. Ensuring compliance with these regulations and conducting extensive safety testing are essential for gaining approval and consumer trust.

#### **4.4 Potential market opportunities for novel fermented tea products**

Despite these challenges, the development of novel fermented tea products through microbial community engineering offers substantial market opportunities. Consumers are increasingly seeking functional beverages that offer health benefits beyond basic nutrition. Engineered fermented teas can be tailored to enhance specific health-promoting properties, such as improved gut health, enhanced metabolic functions, or increased antioxidant levels. Moreover, the ability to modify flavor profiles genetically can attract a broader consumer base, looking for unique and enhanced beverage experiences. As consumer preferences shift towards natural and health-promoting products, novel fermented teas are well-positioned to meet these demands, potentially creating a significant niche in the functional beverages market.

### **5 Future Perspectives**

The development of novel fermented tea products through microbial community engineering is an exciting and rapidly evolving field. As we look to the future, several emerging technologies and approaches hold promise for revolutionizing the way we approach fermentation processes.

#### **5.1 Emerging technologies in microbial engineering and their potential impact**

Recent advances in microbial and mammalian strain development have paved the way for the sustainable production of valuable bio-compounds, biofuels, and biopharmaceuticals (Khaleghi et al., 2021). The integration of advanced processing technologies such as high pressure, electric fields, and ultrasound at sub-lethal levels has shown potential in stimulating microbial growth and manipulating metabolic behavior, leading to fermentation products with novel characteristics (Mota et al., 2018). These technologies, when applied judiciously, could lead to increased yields, productivities, and fermentation rates, as well as the production of different compounds, thereby enhancing the diversity and quality of fermented tea products.

#### **5.2 The role of machine learning and artificial intelligence in predicting and optimizing fermentation outcomes**

Machine learning (ML) has emerged as a powerful tool in systems metabolic engineering, contributing to host strain selection, metabolic pathway reconstruction, metabolic flux optimization, and fermentation (Kim et al., 2020). The synergy between constraint-based modeling (CBM) and ML is particularly noteworthy, as it allows for the creation of more predictable models that can analyze and optimize fermentation parameters (Khaleghi et al., 2021). By leveraging the vast amounts of bio big data, such as omics data, ML can identify patterns within complex biological systems and processes, enabling the rational design of fermentation processes and the prediction of cellular phenotypes from genotypes (Kim et al., 2020). The application of knowledge engineering in conjunction with ML can further enhance the development of quality ML models, providing new constraints for mechanistic models to describe cellular regulations and estimate fermentation outcomes under specific growth conditions (Oyetunde et al., 2018).

#### **5.3 Sustainability and environmental considerations in engineered tea fermentation**

As the demand for sustainable and environmentally friendly production methods increases, microbial fermentation technology must adapt to meet these challenges. The transition to a bio-based society necessitates the acceleration of advancements in microbial fermentation technology, with a focus on the sustainability factor (Begum et al., 2021). The development of novel microbial fermentation processes should consider the environmental impact, aiming to reduce waste and by-product accumulation while maximizing the use of renewable resources. The integration of system biology-led metabolic engineering strategies, genomics, and other omics technologies can aid in the design of efficient and sustainable fermentation processes that align with environmental considerations (Begum et al., 2021).

In conclusion, the future of engineered tea fermentation is bright, with emerging technologies in microbial engineering, the application of machine learning and artificial intelligence, and a strong emphasis on sustainability poised to significantly enhance the field. These advancements will not only improve the quality and variety of fermented tea products but also contribute to a more sustainable and environmentally conscious approach to bio-manufacturing.

## 6 Concluding Remarks

### 6.1 Summary of key findings and discussions

The exploration of microbial communities in the fermentation of various traditional teas has provided significant insights into the complex interactions between microbes and the biochemical changes they induce in tea products. Studies have consistently shown that microbial diversity is a critical factor in the fermentation process, influencing the flavor, aroma, and health benefits of the final tea product.

Integrated meta-omics approaches have advanced our understanding of the microbiota, metabolites, and enzymes involved in the fermentation of Pu-erh tea, revealing a dynamic shift from *Proteobacteria* to *Firmicutes* and the dominance of fungal genera such as *Aspergillus* during different fermentation stages (Zhao et al., 2015; Ma et al., 2017; Zhao et al., 2019). Similarly, the non-filamentous fungi growth-based fermentation of Miang, a traditional fermented tea of North Thailand, has been characterized by a surge in lactic acid bacteria, yeast, and *Bacillus*, with *Firmicutes* and *Ascomycota* being the dominant phyla (Unban et al., 2020).

The solid-state fermentation (SSF) of Post-fermented Pu-erh tea has been shown to involve a succession of microbial communities, with a significant correlation between microbial changes and the dynamics of chemical compounds such as tea polyphenols and catechins (Ma et al., 2017). The role of microbial enzymes in the degradation of plant cell walls and the oxidation of catechins has been highlighted, emphasizing the importance of microbial activity in the soft-rotting of tea leaves (Zhao et al., 2015).

In the context of primary dark tea, the pile-fermentation process has been associated with a shift in microbial communities and the biochemical components they produce, with fungi contributing more significantly to the characteristic properties of the tea than bacteria (Li et al., 2018). The microbial community analysis of Sichuan South-road Dark Tea has identified *Aspergillus niger* as a key player in the development of organoleptic quality during pile-fermentation (Zou et al., 2022).

The microbial community structure and change during the solid fermentation of Pu-erh tea have been studied using PCR-DGGE, revealing a steady microbial community at the last stage of fermentation, with *Aspergillus niger* and *Bacillus* being dominant species (Yang et al., 2013). Kombucha tea fermentation has been investigated for its microbial and biochemical dynamics, showing that the microbial community structure plays a crucial role in the beneficial properties of the beverage (Chakravorty et al., 2016). Lastly, the relevance between bacteria and metabolites in Fuzhuan Brick Tea has been explored, indicating that bacteria are involved in the changes of the metabolomics profile during fermentation (Xia et al., 2021).

### 6.2 Final thoughts on the future of engineered microbial communities in the tea industry

The future of engineered microbial communities in the tea industry looks promising, with the potential to enhance the quality, flavor, and health benefits of tea products. The insights gained from the studies cited above pave the way for the development of novel fermented tea products through microbial community engineering. By manipulating microbial communities, it may be possible to control and optimize the fermentation process, leading to consistent and improved tea products.

The integration of meta-omics techniques will continue to be instrumental in unraveling the complexities of microbial ecosystems in tea fermentation. This knowledge can be applied to select and cultivate specific microbial strains that contribute positively to the fermentation process. Furthermore, the understanding of microbial succession and the dynamics of chemical compounds during fermentation can inform the development of targeted interventions to enhance desirable properties in tea.

As research progresses, there is also an opportunity to explore the commercial application of these findings, potentially leading to the creation of new tea varieties and fermentation methods that cater to the evolving tastes and health-conscious preferences of consumers. The tea industry may witness a revolution where traditional methods are complemented by scientific innovation, ensuring the preservation of cultural heritage while embracing modern technology.

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### Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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