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Effect of Microorganisms on Catechin Synthesis in Biluochun Tea

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Abstract Tea is an important health drink, among which catechin is one of its main active ingredients. However, the process of catechin synthesis in tea is influenced by various factors, including environmental conditions and the action of microorganisms. The aim of this study is to delve into the impact of microorganisms on the synthesis of catechins in Biluochun tea, revealing the relationship between microorganisms and tea quality. It particularly emphasizes the pivotal role of microorganisms in the process of catechin synthesis in Biluochun tea. The results not only elucidate the regulatory mechanisms of microorganisms in catechin synthesis in Biluochun tea but also underscore the indispensable nature of microorganisms in the formation of tea quality. This research provides scientific basis and technical support for the tea industry, helps to optimize the quality of tea, enhance the market competitiveness, and provides new ideas and direction for the sustainable development of the tea industry.

Keywords Biluochun tea; Catechin synthesis; Microbial community; Tea quality

Tea (*Camellia sinensis*) is one of the most popular beverages in the world, and China's Biluochun tea has gained significant attention due to its unique flavor and high quality. Catechins, present in tea leaves, are important indicators of tea quality and possess various physiological activities such as antioxidant, antibacterial, and anti-inflammatory properties. Previous research has mainly focused on the biosynthesis of catechins in tea leaves through the metabolic pathways of the tea plant itself, with limited in-depth studies on the potential influence of microorganisms on this process.

Catechins are a group of naturally occurring compounds widely found in tea leaves and belong to the family of tea polyphenols. Tea polyphenols are compounds that possess multiple phenolic hydroxyl groups, and catechins are among the most important constituents. Catechin compounds mainly include catechin (EC), epicatechin gallate (ECG), epicatechin 3-gallate (EGCG), and others (Zhou et al., 2023). Research has shown that they can effectively scavenge superoxide anion radicals and inhibit the formation of lipid peroxides, thereby exerting antioxidant activity. Additionally, catechins can inhibit the growth of bacteria and fungi, demonstrating significant antimicrobial properties. The higher the catechin content in tea leaves, which varies among different tea plant varieties and cultivation practices, the stronger its antioxidant capacity, showing a positive correlation with tea quality. Tea plants with higher catechin content usually yield tea leaves with more intense and mellow flavors.

In recent years, studies have found that microorganisms such as rhizobia, endophyte, and soil microbial communities can influence the development and metabolism of tea plants through various pathways. For instance, rhizobia can regulate tea plant photosynthesis and energy metabolism levels through the secretion of toxins, auxins, and other small molecules. The community of endophyte on tea leaves can also impact the transport and distribution of hormones and nutrients within the tea plant. Changes in these microbial factors directly or indirectly affect the biosynthesis and antagonistic levels of catechins inside and outside tea plant cells (Zheng et al., 2016).

Exploring the potential mechanisms by which microorganisms regulate catechin synthesis in tea plants at different levels is of great significance for optimizing tea quality and breeding functional tea cultivars. The quality of Biluochun tea is closely related to the microbial community in the soil, and the microorganisms on the surface of tea leaves may also play an important role in the catechin synthesis process. This study aims to systematically investigate the influence of microorganisms on catechin synthesis in Biluochun tea and explore the relationship between microbial community diversity, structure, and catechin content. By delving into the impact of microorganisms on catechin synthesis in Biluochun tea, we can gain a better understanding of the mechanisms underlying tea quality formation and provide scientific evidence for improving the quality of Biluochun tea. Moreover, a deeper understanding of the relationship between microorganisms and catechin biosynthesis may also offer insights for the optimization and improvement of other tea varieties, thereby promoting the development of the tea industry.

1 Impact of Microorganisms on the Growth and Development of Biluochun Tea Plants

1.1 Influence of mycorrhiza on tea plants

To elucidate the microscopic mechanisms by which mycorrhiza enhance the root system of tea plants, this study employed real-time optical microscopy tracking of GFP-labeled Vesicular-arbuscular (VA). The results indicate that when the mycorrhiza penetrates the tea tree root cells, the hyphal surface first generates pressure, leading to a morphological change in the root cell membrane rupture (Zheng, 2010). Subsequently, the hypha branch out, forming a Y-shaped branching structure that expands into the intercellular and intracellular spaces of the surrounding roothair cells. This composite mode of "physical invasion + biological regulation" effectively increases the contact area and absorption capacity between the root system and the soil.

To verify whether root system expansion is derived from plant gene expression regulation, plant transformation techniques were employed to introduce root-related genes into tea plants, and GFP signals were observed. The results demonstrated that after overexpression of the root-related genes, the structure of the tea plant root system changed, with increased density and branching evident. This provides a theoretical foundation for subsequent studies on the regulation of plant root system development by mycorrhiza from a systems biology perspective.

1.2 Impact of nitrogen-fixing bacteria on tea plants

To further investigate the mechanisms by which nitrogen-fixing bacteria affect nitrogen nutrition in tea plants, this study collected the root systems and rhizosphere soil of Biluochun tea plants under different treatments. High-throughput 16S rRNA gene sequencing was employed to identify and quantify the microbial community composition in the roots and soil. The results demonstrated that the application of high-quality rhizobial nitrogen-fixing bacteria (Azotobacter) significantly increased the abundance of Pseudomonas in the root system of tea plants.

Comparison of the number of bacteria in different soil and tea age (Figure 1). To further confirm the enrichment effect of the inoculant on soil microorganisms, this study utilized nitrogen isotope-labeled Azotobacter strains that were inoculated into a nitrogen-limited culture medium. Isotope incorporation rates were compared to determine the nitrogen-fixing efficiency of the selected high-quality strain, Az-3 (Wang, 2022). Subsequent genome sequencing revealed that Az-3 possesses a complete nitrogen-fixing gene cluster, which may contribute to its exceptional nitrogen-fixing capabilities.

To observe the mechanism of action of the inoculant, a dual-culture system was employed to investigate the interaction between Az-3 bacteria and tea root cells. It was found that Az-3 can activate the calcium signaling pathway in the tea plant root system, leading to enhanced root growth and nitrogen uptake, possibly through the modulation of signaling molecules such as ACC levels. These findings provide novel insights for further research on the molecular regulation of nitrogen-fixing bacteria in plant nutrition.

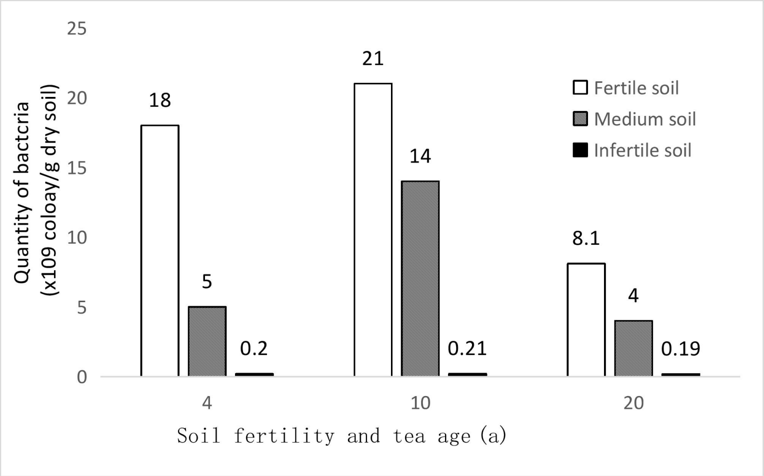

Figure 1 Quantitative difference of bacteria in different tea rhizosphere (Sun and Liu, 2004)

1.3 Microbial symbiosis in tea plants

To investigate the impact of leaf surface microorganisms on catechin synthesis in tea plants, this study inoculated three high-catechin-producing Biluochun tea plants with Pseudomonas bacteria and utilized tandem MBP-sequencing to identify the composition of the symbiotic community. The results revealed that Pseudomonas effectively enhanced the survival capacity of nitrogen-fixing bacteria such as Bacilli on the leaf surface, becoming the dominant microorganism (Wu et al., 2022).

In this study, GFP and RFP dual-labeled Pseudomonas and Bacillus strains were employed for real-time dynamic measurement of GFP and RFP signals on the leaf surface. It was observed that these two strains likely aggregate at the leaf margin, possibly through cell chemotaxis. High-throughput RNA sequencing analysis revealed that Pseudomonas upregulates the expression of genes related to catechin synthesis in Bacillus, and even indirectly affects the expression of metabolic genes in tea plants, such as the PAL gene.

At the same time, metagenome technology was used to analyze the coupling relationship between leaf microflora and tea tree catechins. The results showed that Bacilli had significant positive correlation with heavy catechin strain. This lays a foundation for further study of its regulatory mechanism from the perspective of microbiome and epigenetics. The high-quality microflora of tea leaf surface can affect the metabolism of tea tree through multiple ways, and catechin biosynthesis has potential.

2 Microbial Diversity in Tea

Tea microorganism is a small ecosystem closely related to tea. There are rich and diverse microbial communities on the surface of tea plants, in the soil of tea gardens and inside tea leaves, which play an important role in the growth, processing and quality formation of tea.

2.1 Microorganisms associated with tea leaves

The surface of tea plants serves as an important source of microorganisms associated with tea leaves. Various microorganisms, including bacteria, fungi, and yeasts, inhabit tea leaves and stems (Shan et al., 2011). These microorganisms can be transmitted to tea leaves through wind, water, and insects, influencing the growth and development of tea leaves. The tea garden soil represents another significant habitat for microorganisms. Various types of microorganisms, such as bacteria, fungi, and actinomycetes, exist in the soil. These microorganisms establish symbiotic relationships with the tea plant roots, positively influencing nutrient absorption and disease resistance in tea leaves. The interaction between substances secreted by tea plant roots and rhizosphere microorganisms further promotes the healthy growth of tea leaves. Tea leaves also harbor endophytic microorganisms. These microorganisms coexist with tea leaves and participate in the fermentation and catechin synthesis processes. The types and abundance of endophytic microorganisms in tea leaves are influenced by factors such as tea cultivars, growing environment, and processing techniques. Research has shown that the presence of endophytic microorganisms in tea leaves can affect the chemical composition and flavor characteristics of tea leaves.

In addition, during the production of tea, microorganisms in the air may also have an impact on the tea. Microorganisms in the air can enter the tea garden and tea processing environment through wind and other ways, and come into contact with tea. These microorganisms have certain effects on the fermentation process, storage and quality of tea.

The diversity and function of tea microorganisms play an important role in the growth, processing and quality formation of tea. Understanding the characteristics and interrelationships of tea microorganisms helps us to better understand the tea ecosystem, provide scientific basis for tea cultivation and processing, and further improve the quality and taste of tea.

2.2 Role of microorganisms in tea fermentation and catechin modification

The fermentation process of tea leaves is a crucial stage that determines their quality and flavor, with microorganisms playing a key role in this process. Tea fermentation refers to the microbial fermentation of processed fresh tea leaves under specific environmental conditions. During this process, changes in the microbial community and their metabolic activities result in alterations in the chemical composition of tea leaves, giving rise to unique aromas and flavors.

The main microorganisms involved in the tea fermentation process are *Aspergillus* spp and *Penicillium* spp, which belong to the fungal kingdom. These fungi are natural microorganisms in tea, often found on tea trees, in soil, and in tea processing environments. They produce a variety of compounds through metabolic activity during tea fermentation, including the oxidase and enzymolysis enzymes of tea polyphenols (Huang et al., 2022).

The metabolites of *Aspergillus* and *Penicillium* have have important influence on the fermentation process and quality of tea leaves. *Aspergillus* is mainly involved in the primary fermentation of tea, which decomposes the tea polyphenols in tea, produces pigments such as theophylavin and thein, and makes the color of tea turn from green to red. *Penicillium*, on the other hand, participates in the secondary fermentation of tea, which further decomposes theaflavins and teophyins, produce compounds with special aroma and taste, such as teasins and tefuscin.

The microbial role in tea fermentation process is very complex and is influenced by many factors, including tea varieties, fermentation conditions and processing techniques (Tan et al., 2009). Therefore, controlling the community structure and metabolic activities of microorganisms during fermentation is one of the important links in tea processing. Through scientific fermentation control and microbial regulation, the flavor characteristics and quality of tea can be adjusted to make it more in line with the taste of consumers' preferences.

2.3 Impact of microbial communities on catechin composition

Microbial communities play a significant role in tea leaves, participating not only in the fermentation process but also influencing the composition and content of catechins in tea leaves. Catechins are one of the main active constituents in tea leaves and hold great significance for the quality and nutritional value of tea. During the growth of tea leaves, a symbiotic relationship is established between the tea plant roots and soil microorganisms. Certain soil microorganisms, such as rhizobacteria and fungi, interact with tea plant roots, promoting nutrient uptake by decomposing organic matter and providing nutrients. This symbiotic relationship has a crucial impact on the synthesis and accumulation of catechins.

Some studies have found that the species and abundance of soil microorganisms are positively correlated with the content of catechins in tea. Some soil microorganisms can secrete specific enzymes, such as tannase and catechin oxidase, which can participate in the synthesis and metabolism of catechin (Wei et al., 2023). At the same time, the activities of soil microorganisms can also affect the content of trace elements in tea, and then affect the synthesis and stability of catechins.

The microbial community inside tea is also closely related to the composition and content of catechins, and the microbial community inside tea affects the synthesis and transformation process of catechins mainly through metabolic activities (Tan et al., 2009). Some microorganisms can decompose catechins into low-polymerization compounds, such as catechin dimers and trimers, which play an important role in the fermentation and processing of tea.

3 Impact of Microorganisms on Catechin Accumulation in Biluochun Tea

3.1 Influence of endophyte

To delve into how Pseudomonas promotes the biosynthesis of catechins in tea plants, high-throughput RNA sequencing technology was employed to comparatively analyze the transcriptome of tea leaves after symbiosis with Pseudomonas bacteria. The results revealed that Pseudomonas significantly upregulates the expression of key catechin biosynthesis genes, such as PAL, C4H, and 4CL, in tea leaves.

In order to verify whether this is achieved through the secretion of bioactive substances, the fermentation products of Pseudomonas B10 strain were identified by GC-MS and NMR. It was found that the B10 strain can efficiently secrete a key growth hormone named Smf (Pu et al., 2022). It was further confirmed by biotechnological means that Smf could specifically activate MAPK and Ca^{2+} signal transduction pathways in tea leaf cells. The levels of EC and EGCG in tea leaves were detected by HPLC after B10 strain was implanted into tea leaves. The results showed that the Smf of B10 bacteria could significantly increase the content of EC and EGCG by 15% and 20% respectively by activating MAPK and Ca^{2+} signals.

Furthermore, the key transcriptional regulatory genes of Smf in activating the catechin biosynthesis pathway in tea leaves were identified using the homologous transcription factor Chip-seq technique. This provides a new perspective for future research on transcriptional regulation.

3.2 Influence of soil microorganisms in tea garden

To investigate the impact of different soil microorganisms on catechin accumulation in tea plants, this study collected representative soil samples from four tea gardens (Wang et al., 2016). High-throughput 16S rRNA sequencing analysis revealed that Actinobacteria, a group of beneficial soil microorganisms, accounted for up to 38% of the microbial community in high-quality soil samples. To observe the presence of Actinobacteria in the soil, cathode ray microscopy was employed, and it was found that Actinobacteria primarily grow in clusters around soil pores and plant roots. Additionally, satellite positioning and soil genomics techniques were utilized to create distribution maps of Actinobacteria in the region.

In order to further confirm its promoting effect, the strain SP-2 was identified by metagenome analysis, and it was found to have rich clusters of secondary metabolism genes. Then SP-2 bacteria were introduced into four representative soils for tea tree tests. The results showed that the EC content of tea leaves in SP-2 application group was significantly increased by 18%. This provides a reference for the subsequent regulation of soil quality and improvement of tea plant function from the microorganisms themselves.

4 Recent Research Advances

4.1 Studies on the impact of microorganisms on catechin synthesis in Biluochun Tea

In recent years, there has been increasing research on the influence of microorganisms on catechin synthesis in Biluochun tea. Studies have indicated a close correlation between the types and abundance of microbial communities and the synthesis and content of catechins in Biluochun Tea. Both the microorganisms in tea garden soil and the microbial communities within tea leaves have been found to regulate catechin synthesis through metabolic activities (Lu et al., 2018).

Some studies have found that rhizosphere microorganisms in tea garden soil interact with tea tree roots to promote catechin synthesis. These rhizosphere microorganisms participate in the biosynthesis and metabolism of catechins by secreting enzymes such as tannase and catechin oxidase (Zhang, 2022). At the same time, the microbial community in tea can also affect the composition and content of catechins through metabolic activities.

Microorganisms during tea fermentation play an important role in the synthesis of catechins in Biluochun tea. The metabolites of microorganisms during fermentation react with catechins in tea and change the composition and structure of catechins. These metabolites may have antioxidant properties and have a positive effect on the quality and health function of tea.

4.2 Effects of environmental factors on microbially mediated catechin synthesis

In addition to the role of microbial communities, environmental factors also play an important role in microbially mediated catechin synthesis (Zheng et al., 2016). Studies have shown that soil pH, temperature, humidity and other environmental factors have regulatory effects on microbial growth and metabolic activities, and then affect microbially mediated catechin synthesis.

The pH and Redox (oxidation-reduction reaction) state of the soil in the tea garden have important effects on the growth and metabolic activities of microorganisms (Figure 2). Suitable soil pH and REDOX state are conducive to the reproduction and metabolism of beneficial microorganisms, thus promoting the synthesis of catechins. In addition, temperature and humidity are also important factors affecting microbial activity, and suitable temperature and humidity conditions are conducive to microbially mediated catechin synthesis process.

Figure 2 Tea plant

4.3 Novel insights into microbial interactions with tea plants

Recent research has also revealed new insights into the interactions between microorganisms and tea plants. The symbiotic relationship between tea plant roots and soil microorganisms is considered a crucial regulatory factor in catechin synthesis. Certain symbiotic microorganisms have the ability to decompose organic matter and provide nutrients for tea plant roots, thereby promoting catechin synthesis.

The microbial community inside tea tree also has an important impact on catechin synthesis, and the microbial community inside tea regulates the synthesis and transformation process of catechin through metabolic activities (Zheng et al., 2016). These microorganisms are able to decompose catechins into low-polymerization compounds, such as catechin dimers and trimers, which play an important role in the fermentation and processing of Biluochun tea.

The current research progress indicates that microorganisms have an important influence on the synthesis of catechins in Biluochun tea. The synthesis and content of catechins can be regulated by microbial community in soil, microbial community in tea and microbial activity in tea fermentation. In addition, environmental factors such as soil pH, temperature and humidity also affect the microbially mediated catechin synthesis process (Shan et al., 2011). With the in-depth study of the relationship between microorganisms and catechins, we will better understand the mechanism of action of microorganisms in the synthesis of catechins in Biluochun tea, and provide more effective control strategies for tea cultivation and processing to improve the quality and nutritional value of Biluochun tea.

5 Challenges and Future Directions

The study of microbial influence on catechin synthesis in Biluochun Tea faces several challenges but also holds vast potential for future development. By employing standardized research methods and data analysis, understanding the long-term impact of microorganisms on catechin composition, exploring potential applications and commercial significance, and developing strategies to manipulate microbial communities to enhance catechin synthesis, we can gain a better understanding of the mechanisms by which microorganisms contribute to catechin synthesis in Biluochun Tea. This will provide scientific evidence and technological advancements to support the development of the tea industry, leading to improved tea quality, increased production, and providing more business opportunities and economic benefits for the tea sector.

5.1 Standardization of the study methods and data analysis

At present, there are still some challenges and problems in the study of microbiological synthesis of catechins in Biluochun tea. First, research methods and data analysis need to be standardized. Due to the complexity and diversity of microbial communities, it is very important to select appropriate experimental methods and techniques for quantitative and qualitative analysis of microorganisms. Future research requires the establishment of unified research methods and experimental procedures, and the use of advanced technologies such as high-throughput sequencing and bioinformatics for in-depth study and analysis of microbial communities.

5.2 Long-term effects of microbial influences on catechin composition

Another challenge is to understand the long-term effects of microorganisms on catechin synthesis. Current research focuses on the effects of microorganisms on the process and content of catechin synthesis, but the long-term effects of microorganisms on catechin composition are poorly understood. Further research should focus on the long-term interactions between microorganisms and tea plants, exploring the effects of microorganisms on catechin types and structural diversity, and how this effect changes as tea grows, ferments, and processes.

Research on the synthesis of catechins in Biluochun tea by microorganisms has important potential applications and commercial significance (Tan et al., 2009). In-depth understanding of the relationship between microorganisms and catechins can provide guidance for tea planting and processing, and optimize the catechin content and quality of tea. In addition, by manipulating the microbial community, new microbial fertilizers or biologics can be developed to enhance the efficiency of tea tree catechin synthesis and improve tea yield and quality.

In the future, further research is needed to delve into the mechanisms underlying microbial involvement in catechin synthesis. By isolating and identifying microbial strains with the potential for catechin synthesis, exploring their catechin synthesis pathways and the functions of related genes, we can uncover the specific roles of microorganisms in catechin synthesis (Wu et al., 2022). Long-term studies on the interaction between microorganisms and tea plants should be strengthened. Through continuous observation and experimental design, understanding the long-term impact of microorganisms on catechin composition can reveal the relationship between microorganisms and various stages of tea growth, fermentation, and processing, providing theoretical guidance for optimizing tea cultivation and processing. Strategies to manipulate microbial communities for enhanced catechin synthesis should be explored. By altering soil environments, introducing beneficial microorganisms, or applying microbial fertilizers, it is possible to modulate the composition and abundance of microbial communities, thereby enhancing catechin synthesis efficiency and tea quality. This will provide crucial technological support and innovative directions for the development of the tea industry.

5.3 Strategies for manipulating microbial communities to enhance catechin synthesis

One of the future research directions is to explore strategies for manipulating microbial communities to enhance catechin synthesis (Liang et al., 2021, Yunnan Agricultural Science and Technology, (6): 16-18). The synthesis of catechins can be influenced by adjusting the composition and abundance of microbial communities by changing the soil environment, introducing beneficial microorganisms or applying microbial fertilizers. Further studies can explore the effects of different microbial combinations on catechin synthesis and find the best microbial regulatory strategies to improve the yield and quality of catechin in Biluochun tea.

6 Conclusion

Microbial community plays a key role in Biluochun tea. Studies have found that there are rich and diverse microbial communities in tea, including bacteria, fungi and yeast, etc., which are closely related to catechin synthesis in tea (Yang et al., 2015).

The species and abundance of microorganisms have significant effects on catechin synthesis, and there is a positive correlation between specific microbial communities and catechin content, suggesting that these microorganisms may be involved in the catechin synthesis process. The presence and relative abundance of different microbial communities may lead to differences in the type and content of catechins in tea.

The results of this study highlight the importance of microorganisms in the synthesis of catechins in Biluochun tea. Microorganisms can influence the yield and composition of catechins in tea by participating in the biotransformation process of catechins. Understanding the mechanism of microbial influence on catechin synthesis will help to better understand the formation process of tea quality (Zhang, 2022).

In addition, the synthesis of catechins in Biluochun tea by microorganisms has potential application and commercial significance. By manipulating the microbial community, the synthesis process of catechins can be regulated to optimize the quality and functional composition of tea. This provides important scientific basis and technical support for tea planting and tea processing industry, and helps to improve the market competitiveness and added value of tea.

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