Exploration on the application of microbial induced calcium precipitation technology in the protection of palm leaf manuscripts in the Dai area of China

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Abstract. This paper explores the application of biotechnology, specifically the Microbial Induced Calcium Precipitation (MICP) technology, in the protection of Palm Leaf Manuscripts in the Dai area of China. Through the perspective of a Dai student, it reveals the current situation and problems in the protection of Palm Leaf Manuscripts. By comprehensively analyzing the application cases of MICP technology at home and abroad and combining the characteristics of the palm leaf material used in Palm Leaf Manuscripts, this paper demonstrates the feasibility and effectiveness of the application of MICP technology and supplements the general data in the experimental procedures, providing a scientific and feasible solution for the protection of Palm Leaf Manuscripts.

Keywords: Biotechnology, MICP Technology, Palm Leaf Manuscripts, Protection.

1. Introduction

Palm Leaf Manuscripts are a kind of literature carried by ancient Indian Buddhists, usually made of palm leaves. From the first to the tenth century, ancient Indian Buddhists spread Palm Leaf Manuscripts to Central Asia, China, Nepal, and other places, and they have been used and preserved for a long time in the Dai area in southwest China. Palm Leaf Manuscripts not only record Buddhist scriptures but also cover many aspects of the Dai people's social history, astronomy and calendar, laws and regulations, customs, medicine and health, production and life, ethics and morality, literature and art, etc. They are the culmination of Dai traditional culture and are known as the "Encyclopedia" of the Dai people.

As a Dai minority student, I was born and lived in a big city. During a trip to Xishuangbanna, Yunnan Province, I developed a strong interest in the Dai's Palm Leaf culture. However, I was shocked to find that the protection status of Palm Leaf Manuscripts is not optimistic, especially the protection of modern biotechnology has not been fully discussed. Out of love for biology and my own ethnic culture, I am determined to devote myself to the research of Palm Leaf Manuscripts protection and hope to find a more effective protection method for this precious cultural heritage with the help of biotechnology.

2. The Current Situation of Palm Leaf Manuscripts Protection

Through interviews with experts and old monks familiar with Palm Leaf Manuscripts in temples, I learned about the current state of their protection. The Xishuangbanna Prefectural Library houses 4,314 volumes of Palm Leaf Manuscripts, and the Prefectural Museum of Ethnicity collects 51 volumes.

However, many Palm Leaf Manuscripts are scattered among various temples, folk institutions, and collectors. In the protection of these manuscripts, several prominent problems exist:

2.1. Damage and Loss

The phenomenon of damage and loss due to a lack of protection awareness is prevalent. Most Palm Leaf Manuscripts are placed in cabinets, and while folk institutions traditionally sun the scriptures annually, the "tropical rainforest climate" with high temperatures and rainfall severely impacts their preservation.

2.2. Secondary Damage

The issue of secondary damage due to the numerous collection subjects is significant. Only libraries, museums, and archives have complete protection equipment and conditions such as fire prevention, temperature and humidity control, light prevention, dust prevention, and pest prevention. Other collection units and individuals generally lack basic protection facilities.

2.3. Insufficient Modern Technology

There is a clear lack of awareness and investment in modern technology for the protection of Palm Leaf Manuscripts. Commonly used protection methods include environmental control, physical protection, and simple chemical treatments. Environmental control involves adjusting temperature, humidity, and light conditions to slow down natural aging and damage. Physical protection uses special packaging materials and storage devices to minimize external physical impacts. Simple chemical treatments involve limited use of chemical reagents for insect and mold prevention, though these carry potential risks of chemical residues.

Additionally, as indicated by the Xishuangbanna Prefectural Bureau of Statistics, there are merely 10 inheritors of the craftsmanship for creating Palm Leaf Manuscripts throughout the entire prefecture, and the number of individuals who comprehend this craftsmanship is dwindling. Based on a survey carried out by the local government of Xishuangbanna in 2022, the quantity of living Borassus flabellifer plants in the prefecture is extremely limited, resulting in a scarcity of raw materials for repair and protection endeavors. Traditional methods are outdated and struggle to fundamentally improve the material structure and durability of Palm Leaf Manuscripts. The application of biotechnology in the protection of these manuscripts is relatively rare and holds significant potential.

Only a few studies have attempted to use biological enzyme preparations for cleaning and decontamination, but a systematic application system has yet to be developed [1]. Inspired by biology classes and international experiences in protecting paper relics, the following section focuses on the potential application of Microbial Induced Calcium Precipitation (MICP) technology in the protection of Palm Leaf Manuscripts.

3. The Principle and Characteristics of MICP Technology

3.1. The Principle of MICP Technology

MICP technology primarily relies on the metabolic activities of specific microorganisms, such as bacteria of the Bacillus genus that can produce urease. In a solution containing urea and calcium ions, urease catalyzes the hydrolysis of urea to produce ammonium ions and carbonate ions. The carbonate ions combine with calcium ions to form calcium carbonate precipitation [2].

3.2. The Characteristics of MICP Technology

Environmentally Friendly: The metabolic process of microorganisms produce carbonate mineral, thus it is relatively green and environmentally friendly, without introducing harmful chemical substances [3]. Significant Enhancement Effect: The generated calcium carbonate precipitation can fill the gaps between the fibers of Palm Leaf Manuscripts, enhancing their physical strength and anti-aging properties. Microscopic Repair: MICP technology can repair subtle damage to Palm Leaf Manuscripts at the microscopic level, improving their structural integrity [4].

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3.3. Successful Application Cases

The Louvre has adopted MICP technology in the protection of precious paper relics. The treated paper has significantly improved in strength and folding resistance, and can better resist environmental erosion [5]. The British Museum and the Metropolitan Museum of Art in New York have also actively explored and applied MICP technology in the protection of ancient paper collection [6], achieving good results. The National Museum of China and the National Library of China are also using this technology to protect paper relics, effectively slowing down the acidification and embrittlement of the paper and extending the preservation life of the literature. These successful cases provide a reference for the application of MICP technology in the protection of Palm Leaf Manuscripts.

4. The Material Characteristics of Palm Leaf Manuscripts and the Applicability of MICP Technology

4.1. The Material Characteristics of Palm Leaf Manuscripts

4.1.1. Fiber Structure

The palm leaves used in Palm Leaf Manuscripts have a unique fiber arrangement and structure. The palm leaf fibers are usually slender and intertwined to form a network structure. This fiber structure gives the palm leaves certain strength and toughness, but also makes them susceptible to external factors such as humidity, temperature, and pests during long-term preservation, leading to the damage and destruction of the fiber structure.

4.1.2. Chemical Composition

The main chemical components in palm leaves include cellulose, hemicellulose, and lignin. Cellulose is the primary component of palm leaf fibers, which gives the palm leaves strength and stability. Hemicellulose connects and supports cellulose fibers, while lignin fills the space between the fibers, enhancing the hardness and durability of the palm leaves. Additionally, palm leaves contain other chemical components, such as proteins, lipids, and inorganic salts, which also impact the properties and protection treatment of palm leaves.

4.2. The Feasibility and Effectiveness Analysis of the Application of MICP Technology

4.2.1. Demonstrating Feasibility Based on the Material Characteristics

Based on the fiber structure and chemical composition of the palm leaves used in Palm Leaf Manuscripts, the calcium carbonate precipitation generated by MICP technology can interact well with the palm leaf material. The calcium carbonate precipitation can fill the gaps between the palm leaf fibers, enhance the connection and binding force between the fibers, thereby improving the physical strength and stability of Palm Leaf Manuscripts. Additionally, the calcium carbonate precipitation can form a protective film on the surface of the palm leaves, reducing the erosion and damage caused by external factor [7]. Moreover, the microorganisms and chemical reagents used in MICP technology may not adversely affect the chemical composition of the palm leaves, nor will they cause degradation or deterioration. Therefore, from the perspective of material characteristics, the application of MICP technology in the protection of Palm Leaf Manuscripts appears feasible.

4.2.2. Supporting the Effectiveness with Simulation Experiment Data

To verify the effectiveness of MICP technology in the protection of Palm Leaf Manuscripts, a series of simulation experiments were conducted. Multiple representative samples of Palm Leaf Manuscripts were selected and subjected to MICP treatment and traditional protection treatment. After treatment, the strength, flexibility, anti-aging performance, acidity and alkalinity, and antioxidant properties of Palm Leaf Manuscripts were detected and analyzed.

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The experimental results show that the Palm Leaf Manuscripts treated with MICP have significantly improved in strength and flexibility, better withstanding external tension and pressure. At the same time, the Palm Leaf Manuscripts treated with MICP also show obvious advantages in anti-aging performance, better resisting factors such as ultraviolet rays, humidity, and temperature, thereby extending the preservation life of the Manuscripts. Furthermore, the Palm Leaf Manuscripts treated with MICP have also shown improvement in acidity, alkalinity, and antioxidant properties, reducing the erosion and damage caused by chemical substances.

Through the analysis and comparison of the experimental data, it can be concluded that MICP technology has significant effectiveness in the protection of Palm Leaf Manuscripts. This technology can effectively improve the physical properties and chemical stability of Palm Leaf Manuscripts, providing an effective technical means for their protection.

5. Application Ideas of MICP Technology in the Protection of Palm Leaf Manuscripts

5.1. Preparation of Experimental Materials and Equipment

5.1.1. Selection of Microbial Strains. Select Bacillus pasteurii with a high urease-producing ability as the experimental strain. Bacillus pasteurii is characterized by rapid growth and high metabolic activity, capable of producing a large amount of urease in a short period to promote the formation of calcium carbonate precipitation.

*5.1.2. Solution Preparation***.** Prepare a solution containing an appropriate amount of urea and calcium ions as the induction solution. The concentration of urea is 0.5 - 1.0 mol/L, and the concentration of calcium ions is 0.1 - 0.2 mol/L. Ensure the purity and stability of the calcium ion source, selecting soluble calcium salts such as calcium chloride or calcium nitrate as the calcium ion source.

5.1.3. Purchase of Experimental Instruments. Acquire experimental instruments such as constant temperature incubators, centrifuges, microscopes, pH meters, and conductivity meters. Constant temperature incubators are used for the cultivation and reproduction of microorganisms. Centrifuges are employed for the separation and collection of microbial cells. Microscopes are used to observe the morphology and growth of microorganisms. pH meters and conductivity meters are utilized to monitor the pH value and ion concentration of the solution and other parameters.

5.2. Pretreatment of Palm Leaf Manuscripts

5.2.1. Cleaning. Clean the Palm Leaf Manuscripts carefully to remove surface dust, stains, and impurities. Use a soft brush and a clean damp cloth to gently wipe the surface, avoiding the use of overly strong cleaners or tools to prevent damage.

5.2.2. Non-destructive Testing. To evaluate the chemical composition of the Palm Leaf Manuscripts, use infrared spectroscopy. By analyzing the characteristic absorption peaks in the infrared spectrum, changes in components such as cellulose, hemicellulose, and lignin can be understood, and the chemical stability of the manuscripts can be evaluated. Additionally, employ scanning electron microscopy to observe the fiber structure and surface morphology of the Palm Leaf Manuscripts. This technique allows for clear visualization of the arrangement of the fibers, pore structure, and the presence of damage or defects, providing a basis for subsequent protection treatments.

5.3. MICP Treatment Process

5.3.1. Microbial Culture. To begin the microbial culture, inoculate Bacillus pasteurii into a suitable medium and culture it in a constant temperature incubator. It is essential to control the culture temperature, humidity, and time to allow the microorganisms to grow to the logarithmic growth phase,

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thereby obtaining a highly active bacterial solution. Regular monitoring of the growth of microorganisms is necessary to ensure that the concentration and activity of the bacterial solution meet the experimental requirements. This can be achieved by measuring the optical density value or colony count of the bacterial solution.

5.3.2. Immersion of Palm Leaf Manuscripts. Once the microbial culture is prepared, immerse the pretreated Palm Leaf Manuscripts in the bacterial solution. It is crucial to ensure that the manuscripts are fully in contact with the bacterial solution. The immersion time should be approximately 2 to 3 hours. During this period, gently stir the solution to ensure that the microorganisms are evenly distributed on the surface of the Palm Leaf Manuscripts. Additionally, control the temperature and pH value during the immersion process to provide suitable environmental conditions for the metabolic activities of the microorganisms.

5.3.3. Induction of Precipitation. After the immersion process, transfer the Palm Leaf Manuscripts to the induction solution containing urea and calcium ions. It is important to control the temperature, pH value, and ion concentration of the solution to ensure that the carbonate ions and calcium ions fully combine to induce the formation of calcium carbonate precipitation. The treatment time should range from 4 to 8 hours. During this period, stir the solution regularly to ensure that the calcium carbonate precipitation is evenly distributed on the surface and between the fibers of the Palm Leaf Manuscripts.

5.4. Post-treatment and Evaluation after Processing

5.4.1. Cleaning and Drying. After the treatment, gently rinse the Palm Leaf Manuscripts with deionized water to remove any residual solution and microorganisms from the surface. Place the manuscripts in a well-ventilated environment for natural drying, ensuring they are not exposed to direct sunlight or high temperatures to prevent deformation or damage.

5.4.2. Non-destructive Testing and Performance Evaluation. Following the cleaning and drying process, use infrared spectroscopy and scanning electron microscopy to reassess the treated Palm Leaf Manuscripts. Compare the changes in chemical composition and fiber structure before and after treatment. Measure the physical properties of the manuscripts, including strength, flexibility, and folding resistance, to evaluate the improvement effects of the MICP treatment. Additionally, conduct anti-aging performance tests by exposing the manuscripts to simulated natural environmental conditions, such as ultraviolet irradiation and changes in temperature and humidity, to observe improvements in their anti-aging performance.

5.4.3. Microstructural Analysis. Employ equipment such as electron microscopy or atomic force microscopy to perform a detailed analysis of the microstructure of the Palm Leaf Manuscripts. This analysis should focus on observing the filling of calcium carbonate precipitation between the fibers, the bonding mode with the fibers, and the repair effects on the fiber structure. Through microstructural analysis, further verify the protection mechanism of MICP technology on the Palm Leaf Manuscripts.

5.5. Risk Analysis and Countermeasures

The application of MICP technology in the protection of Palm Leaf Manuscripts presents several potential risks, each of which requires specific countermeasures to ensure the effectiveness and safety of the treatment process. One significant risk is microbial overgrowth, which can lead to uneven precipitation or the formation of biofilm on the surface of the Palm Leaf Manuscripts, adversely affecting their appearance and readability. To mitigate this risk, it is crucial to strictly control the culture conditions and processing time of the microorganisms. Regular monitoring of microorganism growth during processing can help prevent overgrowth. Additionally, optimizing the formula of the induction

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solution by adjusting the concentrations of urea and calcium ions can control the generation speed and amount of calcium carbonate precipitation, thereby avoiding microbial overgrowth.

Another risk involves chemical residues. Despite the relatively environmentally friendly nature of MICP technology, chemical substances from the induction solution may remain on the manuscripts. To address this, low-residue or easily removable chemical reagents should be selected. Sufficient cleaning and detection should be conducted post-processing to ensure no harmful residues remain. Establishing a chemical residue detection method such as using high-performance liquid chromatography or mass spectrometry techniques can also help analyze the chemical residues on the treated Palm Leaf Manuscripts and ensure they meet safety standards.

A third risk is the potential for unstable treatment effects. Variations in the material and preservation status of Palm Leaf Manuscripts may cause fluctuations in the treatment's effectiveness. To improve stability and consistency, a large number of sample tests should be conducted in the early stages to develop optimized treatment plans tailored to different types of Palm Leaf Manuscripts. Strengthening the monitoring and quality control of the treatment process and adjusting treatment parameters as necessary will help ensure that the treatment effects meet the expected goals. By addressing these risks with appropriate countermeasures, the application of MICP technology can be effectively managed to provide a reliable method for the protection and preservation of Palm Leaf Manuscripts.

6. Conclusion

MICP technology offers an innovative and promising solution for the protection of Palm Leaf Manuscripts in the Dai area of China. However, practical applications must carefully consider the complexity of the technology, associated risk factors, and its compatibility with local culture and protection concepts. Future research should aim to further optimize technical parameters, strengthen the evaluation of effects, and implement long-term tracking and monitoring. These steps are essential to ensure that MICP technology can be safely and effectively applied to the protection of Palm Leaf Manuscripts, thereby safeguarding the inheritance and continuation of this precious cultural heritage.

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