Structure Analysis of Culturable Bacterial Communities in Areca Palms Affected by Yellow Leaf Disease

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Abstract [**Objectives**] The paper was to analyze the structure of the culturable bacterial communities in healthy areca palms and those affected by yellow leaf disease (YLD). [**Methods**] The quantification and isolation of culturable bacteria present in the leaves, roots, and rhizosphere soil of both healthy areca palms and those exhibiting symptoms of YLD within the same orchard were conducted. Furthermore, a differential analysis of the bacterial community structure was conducted. [**Results**] The bacterial count was observed to be greater in healthy areca palm samples compared to those exhibiting disease symptoms. Furthermore, the diversity of bacterial species in healthy areca palm samples surpassed that found in diseased samples. Notably, the bacterial genera that exhibited significant differences between healthy and diseased areca palms included *Bacillus velezensis*, *Paenibacillus validus*, *Alcaligenes faecalis*, *Burkholderia territorii*, *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *etc.* [**Conclusions**] This study may offer data support and technical guidance for the effective prevention and control of YLD in areca palm in the future.

Key words Yellow leaf disease (YLD); Culturable bacteria; Structural analysis

1 Introduction

Areca palm (*Areca catechu* L.) is a perennial plant belonging to the Palmae family, thriving optimally in conditions characterized by elevated temperatures and high humidity[1]. Areca palm is not only an important medicinal plant but also a representative species of tropical landscape trees, recognized as one of the four major southern medicinal plants in China[2]. Traditional Chinese medicine holds that the areca nut possesses properties that can eliminate insects, alleviate stagnation, reduce qi, and promote the movement of qi. Appropriate consumption of areca nut is considered beneficial to overall health[3]. Yellow leaf disease (YLD) is a destructive and contagious disease that leads to considerable reductions in yield among areca palms. Endophytes and growth-promoting bacteria present in the rhizosphere soil can enhance disease resistance in plants or induce systemic resistance through various mechanisms, including nitrogen fixation, phosphorus solubilization, phytohormone synthesis, inhibition of pathogen growth, and antibiotic production, all of which contribute to the protection of plants against diseases[4]. Citrus Huanglongbing[5], characterized by its rapid spread, destructive nature, and difficulty in management, has been chosen as the focus of analysis by Liu Bo *et al.* [6] using the PCC method to investigate its relationship with citrus endophytes. Their findings indicated that *Bacillus* sp. and *Bacillus pumilus* exhibited a significant negative correlation with the pathogen responsible for citrus Huanglongbing. Trivedi *et al.* [7] identified that certain citrus endophytic bacteria, including *Bacillus lichenformis* and *Paenibacillus validus*, exhibited a significant inhibitory effect on the occurrence of citrus Huanglongbing. Zhang *et al.* [8] isolated TF28 (*Bacillus amyloliquefaciens*) from soybean and discovered that it not only impeded the mycelial growth of *Fusarium nwnilifornu* in rice, but also inhibited the growth of other pathogenic bacteria. This study aims to quantitatively analyze and identify the culturable bacteria present in the leaves, roots, and rhizosphere soil of both healthy and diseased areca palms. Additionally, a comparative analysis of the bacterial community structure was conducted to elucidate the differences between the bacterial communities associated with healthy and diseased areca palms. The findings of this research are intended to provide valuable data and technical guidance for the effective prevention and control of YLD in areca palm in the future.

2 Materials and methods

2.1 Materials Samples were collected from Baoting, Qionghai, and Wanning in Hainan Province. Areca palms of the same age exhibiting symptoms of YLD were randomly selected, and samples of leaves, roots, and rhizosphere soils were obtained from these affected trees. Additionally, samples from healthy trees, which displayed no evidence of phytoplasma infection, were collected from the same orchard, located within 500 m of the sampled diseased trees, to serve as a control. Each tree was treated as an individual replication, with three replications conducted for both the diseased trees infected with YLD and the healthy trees.

2.2 Methods

2.2.1 Determination of bacterial count. The dilution plate method was employed for the preparation of bacterial suspensions from soil, while a tissue homogenization technique was utilized for leaves and roots. The bacteria were cultured to ascertain the optimal dilution concentration, following the methods established by Xu Huamin[9] and Luo Fei[10]. Subsequently, the number of bacterial

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colonies was quantified.

Number of colonies per gram of soil = Mean number of colonies × 20 × Dilution factor × (Fresh soil weight/Dry soil weight) (1)

Number of strains per gram of tissue = $(C \div V) \times M$ (2)

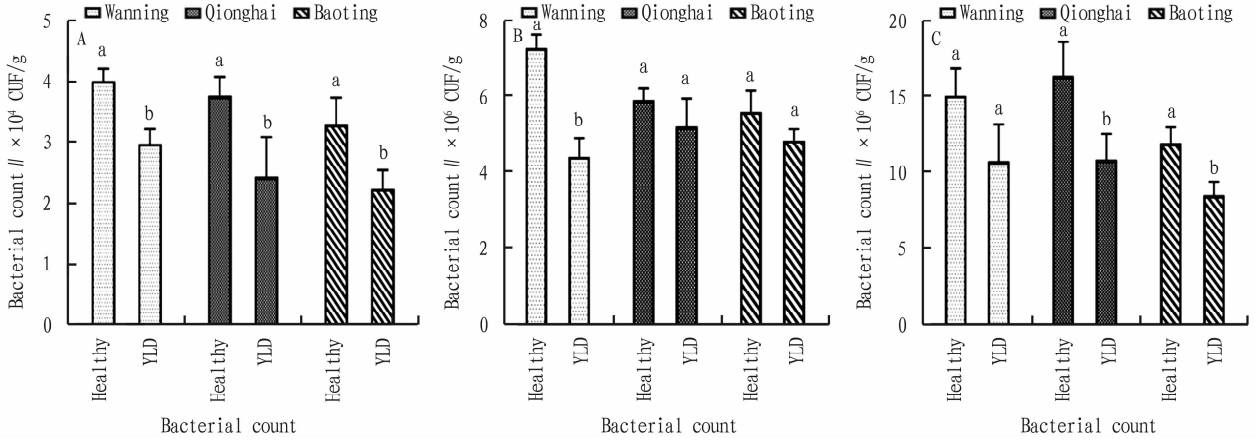
where C denotes the mean number of colonies, V represents the volume of diluent utilized for coating (mL), and M signifies the optimal dilution factor.

2.2.2 Identification of bacterial species. Following the purification of the strains, they were transferred to nutrient agar (NA) plates and incubated at 32 ℃ for 24 h. Subsequently, the transparency, morphology, size, edge, and other characteristics of the colonies were observed. The physiological and biochemical characteristics of the strains were assessed using biochemical test reagents produced by Qingdao Hopebio Co., Ltd. The bacterial strains was identified in accordance with the *Handbook of Systematic Identification of Common Bacteria*[11]. Following the extraction of the strain DNA, the target fragments were amplified, sequenced, and subsequently analyzed through sequence comparison.

3 Results and analysis

3.1 Screening for optimal dilution suitable for counting Following the gradient dilution of the bacterial solutions from each sample, the results of the plate cultures indicated that the optimal dilution for rhizosphere soil bacterial colony counts was 10^4. Similarly, the optimal dilution for root endophytic bacterial colony counts was also 10^4, while the optimal dilution for leaf endophytic bacterial colony counts was determined to be 10^2.

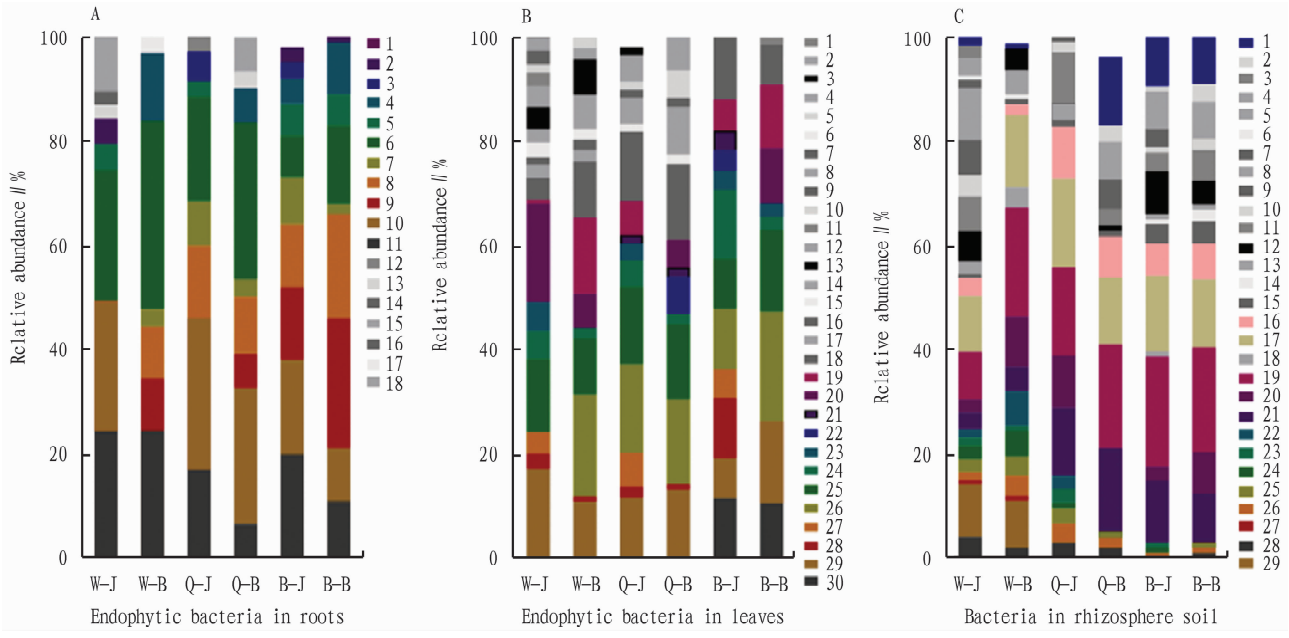
3.2 Statistics on total bacterial counts in healthy and diseased areca palm samples The analysis of bacterial counts in various samples (Fig. 1) revealed that the quantity of endophytic bacteria present in healthy areca palm leaves from Wanning, Qionghai, and Baoting was significantly greater than that found in diseased plants. Additionally, the endophytic bacterial counts in healthy areca palm roots from Wanning were markedly higher compared to those in diseased plants, while the counts in healthy roots from Qionghai and Baoting also exceeded those in diseased plants. Furthermore, the bacterial counts in the rhizosphere soil of healthy areca palms from Qionghai and Baoting were significantly elevated relative to the rhizosphere soil of diseased plants. In Wanning, the bacterial counts in the rhizosphere soil of healthy areca palms surpassed those in the rhizosphere soil of diseased plants.



NOTE A. Endophytic bacterial count in leaves; B. Endophytic bacterial count in roots; C. Bacterial count in rhizosphere soil. Different lowercase letters signify a statistically significant difference at the 0.05 level.

Fig. 1 Comparison of bacterial counts in healthy and diseased areca palm samples in different regions

3.3 Analysis of bacterial diversity in healthy and diseased areca palm samples Following an analysis conducted using BLAST comparison within the NCBI database, as illustrated in Fig. 2, 12 genera were identified in the samples collected from Wanning, Qionghai, and Baoting. The results of the sequencing analysis indicated that the bacterial diversity in the healthy samples was greater than that observed in the diseased samples. The identified genera include *Serratia* sp., *Alcaligenes*, *Pseudomonas* sp., *Bacillus*, *Ochrobactrum* sp., *Pandoraea* sp., *Microsporum*, *Brevibacterium*, *Burkholderia cepacia* sp., *Microbacterium* sp., *Enterobacter* sp., and *Burkholderia* sp. The predominant genera identified in both healthy and diseased samples included *Microbacterium* sp., *Pseudomonas* sp., and *Serratia* sp. In the leaf tissues, the strains that were exclusive to healthy tissues or exhibited significant differences from those in diseased tissues across all three locations were *Pseudomonas aeruginosa*, *Bacillus velezensis*, *Brevibacterium marinum*, and *Alcaligenes faecalis*. In the root tissues, the strains that were specific to healthy tissues or exhibited significant differences from the diseased tissues at three locations included *Bacillus*, *Microsporum*, *Burkholderia territorii*, *Bacillus amyloliquefaciens*, and *B. velezensis*. In the rhizosphere soil, the strains that were specific to healthy tissues or demonstrated significant differences from the diseased tissues across three locations included *Bacillus licheniformis*, *Ochrobactrum* sp., *Paenibacillus* sp., *B. amyloliquefaciens*, *B. territorii*, *B. marinum*.



NOTE W-J. Wanning-healthy; W-B. Wanning-YLD; Q-J. Qionghai-healthy; Q-B. Qionghai-YLD; B-J. Baoting-healthy; B-B. Baoting-YLD. A. 1 – 18. 1. Other; 2. *Pseudomonas aeruginosa*; 3. *Alcaligenes faecalis*; 4. *Ochrobactrum* sp. ; 5. *Bacillus velezensis*; 6. *Microbacterium* sp. ; 7. *Bacillus pumilus*; 8. *Brevibacterium* sp. ; 9. *Alcaligenes aquatilis*; 10. *Serratia* sp. ; 11. *Pseudomonas* sp. ; 12. *Bacillus weihenstephanensis*; 13. *Pandoraea* sp. ; 14. *Burkholderia*; 15. *Bacillus* sp. ; 16. *Brevibacterium marinum*; 17. *Bacillus cereus*; 18. *Bacillus altitudinis*; B. 1 – 30. 1. *Alcaligenes aquatilis*; 2. *Bacillus aerius*; 3. *Sporosarcina newyorkensis*; 4. *Burkholderia*; 5. *Microbacterium nematophilum*; 6. *Microsporum*; 7. *Bacillus haynesii*; 8. *Burkholderia cepacia* sp. ; 9. *Alcaligenes*; 10. *Paenibacillus* sp. ; 11. *Bacillus aryabhattai*; 12. *Bacillus altitudinis*; 13. *Bacillus cereus*; 14. *Ochrobactrum* sp. ; 15. *Bacillus weihenstephanensis*; 16. *Bacillus pumilus*; 17. *Pandoraea* sp. ; 18. *Brevibacterium* sp. ; 19. Other; 20. *Ochrobactrum* sp. ; 21. *Pseudomonas taiwanensis*; 22. *Alcaligenes faecalis*; 23. *Bacillus velezensis*; 24. *Pseudomonas aeruginosa*; 25. *Enterobacter* sp. ; 26. *Microbacterium* sp. ; 27. *Bacillus amyloliquefaciens*; 28. *Bacillus* sp. ; 29. *Serratia* sp. ; 30. *Pseudomonas* sp. ; C. 1 – 29. 1. Other; 2. *Alcaligenes faecalis*; 3. *Brevibacterium marinum*; 4. *Bacillus licheniformis*; 5. *Microsporum*; 6. *Bacillus haynesii*; 7. *Burkholderia cepacia* sp. ; 8. *Pseudomonas* sp. ; 9. *Alcaligenes*; 10. *Paenibacillus* sp. ; 11. *Ochrobactrum* sp. ; 12. *Ochrobactrum* sp. ; 13. *Bacillus weihenstephanensis*; 14. *Bacillus pumilus*; 15. *Pandoraea* sp. ; 16. *Brevibacterium* sp. ; 17. *Serratia* sp. ; 18. *Pseudomonas taiwanensis*; 19. *Microbacterium* sp. ; 20. *Pseudomonas aeruginosa*; 21. *Enterobacter* sp. ; 22. *Bacillus velezensis*; 23. *Bacillus amyloliquefaciens*; 24. *Bacillus subtilis*; 25. *Bacillus cereus*; 26. *Bacillus altitudinis*; 27. *Margalitia*; 28. *Bacillus aryabhattai*; 29. *Bacillus*.

Fig. 2 Species abundance at the bacterial genus level

4 Discussion

The incidence of plant diseases is closely linked to the population dynamics, composition, and functional attributes of microorganisms. These factors ultimately influence the stability of microorganisms. Therefore, the restoration of a stable microbial community is of greater significance than merely managing the occurrence of diseases[12-13]. In this experiment, the differences in microbial counts and diversity between healthy and diseased areca palm samples were preliminarily analyzed through the isolation and identification of culturable microorganisms. The findings of this study regarding the differences between healthy and diseased areca palms are largely consistent with the results reported by Yu Fengyu *et al.*[14]. Both studies indicated that the bacterial counts in healthy samples were higher than those in diseased samples, and that the diversity of microorganisms in healthy samples exceeded that of diseased samples. The current study also examined the structural composition of endophytic bacteria and rhizosphere soil bacteria associated with the areca palm. While there was minimal variation observed at the phylum level, more significant differences were noted at the genus level. The isolation and identification of culturable bacteria revealed a significant disparity between healthy and diseased samples, including species such as *A. faecalis*, *P. aeruginosa*, *B. amyloliquefaciens*, *B. territorii*, *etc.* Notably, *A. faecalis* and *P. aeruginosa* demonstrated enhanced disease-resistant and growth-promoting capabilities in our previous studies. In addition, the genera *Bacillus* and *Pseudomonas* are recognized as prevalent genera of biocontrol bacteria[15]. Huang Jiefang[16] identified that *B. territorii* exhibited a significant inhibitory effect on shoot dieback of pines, with a clearly defined inhibitory zone. Additionally, Sun Zhengxiang *et al.*[17] evaluated the efficacy of the biocontrol bacterium *B. territorii* against watermelon wilt disease, reporting an effectiveness rate of 68.4%. Furthermore, *B. territorii* demonstrated a notable capacity to promote plant growth. The limited capacity to culture microorganisms necessitates the use of advanced technologies, such as amplicon sequencing, to obtain more precise and detailed data.

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(To page 22)

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