Screening of Growth-Promoting Strains for Areca Palm

Dejie YANG△, Yenan WANG△, Zhaowei LIN*, Xiaoqing NIU*

Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences/Hainan Engineering Research Center of Arecanut Industry, Wenchang 571339, China

Abstract [**Objectives**] The paper was to identify growth-promoting strains within the culturable bacterial flora of areca palm. [**Methods**] Culturable bacteria were isolated and identified from areca palm using samples obtained from both healthy and yellowing disease-affected plants within the same orchard. Strains that exhibited significant differences between healthy and affected samples, or that were unique to the healthy samples, were subsequently screened for their growth-promoting effects. [**Results**] Three bacterial strains demonstrated robust and consistent capacity for auxin production, specifically *Paenibacillus*, *Pseudomonas aeruginosa*, and *Bacillus amyloliquefaciens*, each yielding approximately 50 μg of IAA per mL of bacterial solution. The strain *Alcaligenes faecalis* exhibited the highest efficacy in siderophore production, achieving 21.15% of active units. Additionally, *A. faecalis*, *Bacillus velezensis*, and *P. aeruginosa* were noted for their potassium-solubilizing capabilities, as evidenced by the presence of distinct potassium-solubilizing zones. [**Conclusions**] The evaluation of the aforementioned growth-promoting strains may offer valuable insights for the development of growth-promoting strains specifically for areca palm.

Key words Areca palm; Yellow leaf disease; Auxin production; Siderophore production; Potassium-solubilizing

1 Introduction

Areca palm (*Areca catechu* L.) is a perennial evergreen tree belonging to the Palmae family. It is a prominent tropical cash crop, enjoying the reputation of "small areca palm, big industry". The areca palm industry serves as a crucial pillar of the economy in Hainan Province, and its significance in the strategy for precise poverty alleviation is increasingly recognized. In recent years, the phenomenon of yellowing in areca palms has emerged as a serious concern, affecting nearly all cities and counties within Hainan Province. In addition to the pathological yellowing induced by phytoplasma and other factors, physiological yellowing resulting from phytotoxicity and nutritional deficiency also poses a significant threat to the healthy development of the areca palm industry. Yellow leaf disease (YLD) has the potential to induce a yield reduction of 70% - 80% or even result in complete crop failure of the areca palm in regions where the disease is prevalent. Currently, there are no effective control measures available to mitigate its impact. Soil microorganisms constitute a fundamental component of the soil ecosystem and are essential for the self-healing processes of soil[1]. They contribute significantly to the decomposition of organic matter, enhancement of plant nutrient uptake, and regulation of plant diseases[2]. The rhizosphere soil refers to the soil located within the tillage layer, specifically at depths of 5 - 20 cm adjacent to plant roots[3]. Prior research has demonstrated that pathogenic bacteria that infest plants can alter the microbial community structure of plants[4]. Wang Aihua *et al.*[5] conducted a study on healthy soils and soils infected by tobacco bacterial wilt, revealing that healthy soils demonstrated a greater diversity of bacteria. Wang Fang *et al.*[6] conducted a study on the structure of endophytic bacterial communities present in the tissues of citrus plants affected by huanglongbing. Their findings revealed a negative correlation between the abundance of *Serratia marcescens* and the population size of *Candidatus* Liberibacter asiaticus. Liu Bo *et al.*[7] demonstrated that the presence of *Serratia plymuthica*, *Bacillus licheniformis*, *Pseudomonas putida*, *Bacillus fusiformis*, and *Paenibacillus* in the roots of citrus plants led to a significant decrease in the population of *Ca*. Liberibacter. Xiong Dawei *et al.*[8] discovered that the predominant endophytic bacteria in citrus plants affected by huanglongbing exhibited significant changes. Specifically, healthy plants were characterized by the presence of the dominant genera *Granulicella* and *Defluviicoccus*, which were absent in diseased plants. Zhang *et al.*[9] isolated TF28 (*Bacillus amyloliquefaciens*) from soybean and discovered that it not only inhibited the mycelial growth of *Fusarium nwniliformu* in rice plants but also suppressed the growth of other pathogenic bacteria. The utilization of endophytes and rhizosphere soil microorganisms as potential biocontrol agents and exogenous gene carriers in agricultural production presents significant prospects and has emerged as a focal point of research among agricultural experts[10]. This study was conducted to identify beneficial growth-promoting strains from the culturable bacterial flora of areca palm, based on findings from previous research indicating that most differential strains of healthy and diseased areca palms originate from bacterial flora. The aim is to provide guidance for the development of growth-promoting bacterial agents for areca palm cultivation.

2 Materials and methods

2.1 Materials Baoting, Qionghai, and Wanning, identified as the regions most severely affected by YLD in Hainan Province, were selected as sampling sites. Areca palms of the same age exhibiting symptoms of YLD were randomly chosen, and samples of leaves, roots, and rhizosphere soils were collected from these dis-

Received: June 22, 2024 Accepted: September 29, 2024

Supported by Specific Research Fund of the Innovation Platform for Academicians of Hainan Province (YSPTZX202151).

△These authors contributed equally to this work.

*Corresponding author. E-mail: linzhaowei163@163.com; xiaoqingniu123@126.com

eased trees. Additionally, samples from healthy trees, which showed no evidence of phytoplasma infection, were obtained from the same orchard, ensuring that both the management practices and soil texture were consistent (within 500 m of the sampled diseased trees) to serve as a control group. Each tree served as a replication, with three replications for both diseased trees infected with YLD and healthy trees.

2.2 Methods

2.2.1 Isolation of bacterial strains. (i) The dilution plate method was utilized for the analysis of soil samples. The collected and processed soil samples, weighing 10 g, were transferred into a wide-mouth bottle and supplemented with 90 mL of sterile water. The mixture was then incubated on a shaker for 30 min, after which it was diluted to create a bacterial suspension ranging from 10^{-1} to 10^{-6}.

(ii) The tissue homogenization method was employed for the analysis of various tissues, including leaves and roots. In the context of aseptic operations, the experimental materials were subjected to surface sterilization prior to use. The roots were sectioned into strips approximately 0.2 cm in length, while the leaves were diced into pieces measuring 0.5 mm^2. A weight of 0.1 g of the prepared material was then placed into a 2 mL centrifuge tube, to which 1 mL of sterile water and two sterilized steel beads were added. The mixture was subsequently shaken for 4 min using a 120 Hz oscillator, resulting in the preparation of a stock solution of the bacterial suspension. A gradient dilution was subsequently performed to create a bacterial suspension ranging from 10^{-1} to 10^{-6}.

(iii) The microorganisms were cultured to determine the optimal dilution concentration, following the methodologies established by Xu Huamin[11] and Luo Fei[12].

2.2.2 Purification and preservation. All colonies on the optimal dilution plate were selected and subsequently transferred to the corresponding medium for numbered culture through streaking. The colonies were purified through 2 – 3 rounds of purification to isolate pure bacterial strains, which were then preserved using the cryopreservation method.

2.2.3 Bacterial morphology and physiological and biochemical measurements. Following the purification of the strains, they were transferred to nutrient agar (NA) plates and incubated at 32 ℃ for 24 h. The transparency, morphology, size, edge, and other characteristics of the colonies were subsequently observed. The physiological and biochemical characteristics of the strains were assessed utilizing biochemical test reagents manufactured by Qingdao Hopebio Co., Ltd. The identification of the strains was conducted with reference to the *Handbook of Systematic Identification of Common Bacteria*[13].

2.2.4 Molecular identification of strains. Bacterial DNA was extracted using extraction kits and subsequently amplified following quality control measures (primers: 27F/1492R). After the amplification products were assessed for quality through electrophoresis, we commissioned sequencing to Sangon Biotech Co., Ltd. The sequencing results were analyzed using BLAST and identified according to their homology[14].

2.2.5 Screening of growth-promoting strains. (i) Screening of IAA secreting strains. In accordance with the experimental methodology established by Wang Jun[15], the study was conducted through both qualitative and quantitative experiments. Initially, purified strains obtained from healthy samples were inoculated into King's liquid medium and incubated at 28 ℃ with a shaking speed of 180 r/min for a duration of 4 d. Subsequently, equal volumes of bacterial suspensions and Salkowski's colorimetric solution were combined and allowed to undergo dark incubation on white ceramic plates for 30 min. The resulting color changes were then observed; the brightness of the color was used as an indicator to assess the strain's ability to produce IAA. Furthermore, the auxin content produced by the strain was quantitatively determined based on the established standard curve of IAA.

(ii) Screening of siderophore production strains. In accordance with the methodologies established by Liu Lihui *et al.*[16] and Yang Yanan[17], the purified strains obtained from healthy samples were initially inoculated into MSA-CAS liquid medium. The cultures were then incubated for a duration of 2 d at a temperature of 28 ℃ and a shaking speed of 120 r/min. The color change of the medium from blue to red or orange-yellow served as an indicator of siderophore production. A bacterial seed solution with qualitative siderophore production was inoculated into MSA liquid medium at an inoculum size of 1%. A control group was established, and the medium was incubated at 37 ℃ with a shaking speed of 180 r/min for a duration of 3 d. Following incubation, the fermentation broth was subjected to centrifugation, and an equal volume of the fermentation supernatant was mixed with the CAS assay solution. The absorbance of the resulting solution was measured after 1 h and recorded as *As*.

The absorbance value in the control group was denoted as *Ar*, which was calculated using the following formula.

$$\text{Siderophore activity unit (\%)} = [(Ar - As)/Ar] \times 100\% \quad (1)$$

(iii) Screening of potassium-solubilizing strains. 10 μL of bacterial solution derived from the strain obtained from healthy samples was inoculated onto potassium feldspar medium and incubated at a temperature of 28 ℃ for a duration of 2 – 5 d. Strains exhibiting distinct transparent halos indicative of potassium solubilization were classified as positive potassium-solubilizing strains. Highly efficient potassium-solubilizing bacteria were identified based on the presence or absence of a transparent zone surrounding the colony, as well as the magnitude of the solubility index, which was determined following the methodology outlined by Cui Yongliang *et al.*[18].

$$\text{Solubility index} = \text{Hydrolysis zone radius } (R)/\text{Colony radius } (r) \quad (2)$$

3 Results and analysis

3.1 Isolation and identification of culturable bacteria from healthy and diseased areca palm samples Gradient dilutions of

the bacterial solution in each sample were conducted to ascertain the optimal dilution of 10^4 for rhizosphere soil bacterial colony counts, the optimal concentration of 10^4 for root endophytic bacterial colony counts, and the optimal dilution of 10^2 for leaf endophytic bacterial colony counts. The bacterial strains were isolated and purified based on optimal dilution concentrations, resulting in a total of 329 bacterial strains. These strains underwent both biological and analytical identification.

3.2 Screening of auxin production strains As illustrated in Figs. 1 – 2, the intensity of IAA production capacity was assessed in the secretions of over 300 bacterial strains isolated from healthy samples, based on the degree of color change observed during the initial screening reaction. Among these strains, 20 were found to produce auxin. Following additional quantitative screening and verification, three strains exhibiting robust and stable auxin production capacity were ultimately identified. The auxin production capacity of each strain is illustrated in Fig. 2. Strain j3-lv-32 (*Paenibacillus*) exhibited the highest IAA production capacity, achieving a concentration of 61.3 μg/mL. This was followed by strain j3-gl-3 (*B. amyloliquefaciens*) with a concentration of 56.9 μg/mL, and strain wrj-2-5 (*Pseudomonas aeruginosa*) with a concentration of 49.6 μg/mL. The IAA production capacities of these three strains were notably robust, each reaching approximately 50 μg/mL.

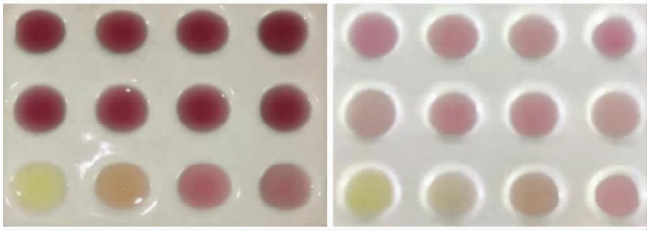

Fig. 1 Color reaction of IAA production by each strain

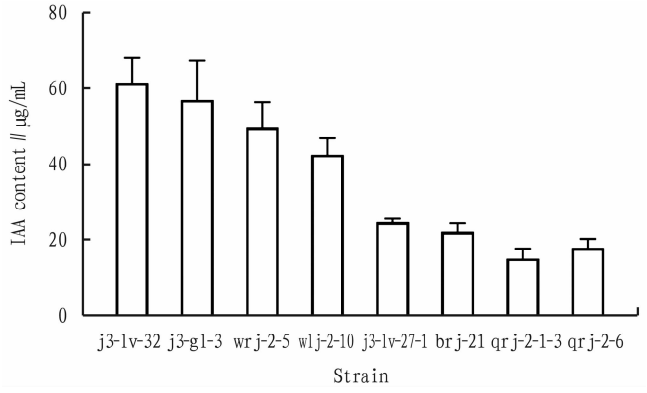

Fig. 2 IAA content produced by each strain

3.3 Screening results of siderophore-producing strain As illustrated in Fig. 3 and Table 1, the results of the initial screening qualitative experiments led to the selection of six strains exhibiting superior siderophore production for subsequent quantitative analysis. The yield of siderophore was assessed using a SA iron-limited culture medium. As illustrated in Fig. 3, the strain brj-21 (*Alcaligenes faecalis*) exhibited the highest yield of siderophore, achieving 21.15% of activity units. Conversely, the strain wsj-31 (*Bacillus velezensis*) demonstrated the lowest yield, attaining 16.94% of activity units for siderophores.

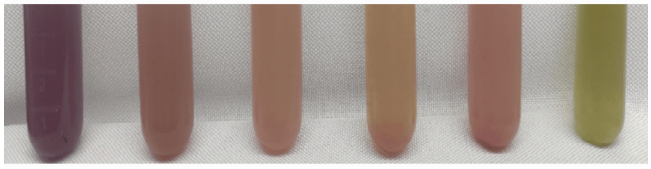

Fig. 3 Chromogenic reaction of siderophore production of various strains

Table 1 Siderophore yield of various strains

Strain	Siderophore yield // %
j3-lv-32	20.48 ± 2.79
wrj-2-5	17.80 ± 2.53
j3-gl-3	19.08 ± 1.14
qrj-2-6-2	18.36 ± 2.87
brj-21	21.15 ± 1.68
wsj-31	16.94 ± 2.36

3.4 Screening of potassium-solubilizing strains As illustrated in Fig. 4 and Table 2, the isolated and purified strains obtained from healthy samples were individually inoculated onto potassium feldspar plates. Following incubation, the presence of a transparent potassium-solubilizing zone surrounding the bacterial colonies was assessed. As a result of the investigation, three strains were identified as exhibiting potassium-solubilizing zones: j3-lv-27-1 (*B. velezensis*), brj-21 (*A. faecalis*), and wrj-2-5 (*P. aeruginosa*). According to the solubility index, the strain brj-21 demonstrated the highest potassium solubilizing capacity, followed by j3-lv-27-1 and wrj-2-5.

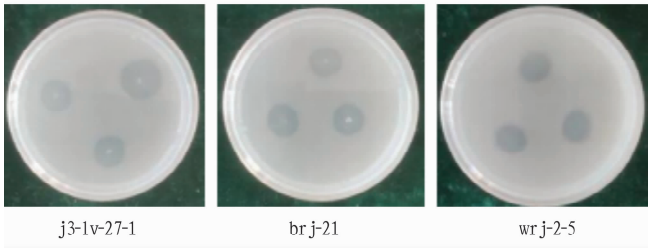

Fig. 4 Potassium-solubilizing zones of potassium-solubilizing strains

Table 2 Screening of potassium-solubilizing strains

Strain	Hydrolysis zone radius (R) // mm	Colony radius (r) // mm	Solubility index (R/r)
j3-lv-27-1	8.27	2.20	3.96
brj-21	9.00	2.27	3.98
wrj-2-5	8.17	2.50	3.17

4 Discussion

In recent years, issues such as soil compaction and nutrient imbalance have arisen due to factors including soil fertility, inadequate management practices by farmers, and the improper application of chemical fertilizers and pesticides. These challenges have ultimately resulted in a decline in areca palm yield and an increased

incidence of diseases. The utilization of plant-promoting and disease-resistant bacteria presents a promising strategy for mitigating these problems[19]. Siderophores can chelate insoluble Fe^{3+} in the soil by forming complexes, which facilitates plant uptake and utilization. This process enhances the iron content within the plant and promotes plant growth[20]. Wang Huan *et al.*[21] isolated *Pseudomonas hunanensis*, which is capable of producing siderophores, from the rhizosphere soil of tea trees. Their findings indicated that this bacterium significantly enhanced both the plant height and fresh weight of water spinach. Potassium-solubilizing bacteria possess the capability to transform mineral potassium present in the soil into a soluble form that can be easily assimilated by plants, thereby enhancing potassium utilization[22]. Song Cong *et al.*[23] isolated a strain of *Sinorhizobium meliloti*, a bacterium known for its efficient potassium-solubilizing capabilities, from mountainous soils in Hebei and Shanxi. Their findings indicated that this strain significantly enhanced the germination rate, biomass, and overall quality of cucumber plants. Jaisingh *et al.*[24] isolated strains of *Bacillus* spp., *Azotobacter* spp., and *Pseudomonas* spp. from sesame soil. These strains were found to significantly enhance sesame plant height, increase the number of branches, and improve the yield per plant compared to the control group. In this experiment, we screened healthy areca palm samples for strains exhibiting enhanced growth-promoting effects. The auxin production strains identified include *Paenibacillus*, *B. amyloliquefaciens*, and *P. aeruginosa*, which produced approximately 50 μg of IAA per mL of bacterial solution. This production level places these strains in the upper-middle range of IAA production capacity when compared to the majority of IAA production strains that have been isolated to date[15, 25]. The most effective siderophore production strain identified in this study was *A. faecalis*, which demonstrated an ability to achieve 20.15% of active units. Notably, Yang Yanan[17] isolated *A. faecalis* from the rhizosphere of tomato plants, which exhibited an even higher capacity of 69.27% of active units. Furthermore, the auxin-producing *P. aeruginosa* and the siderophore-producing *A. faecalis* in this investigation also displayed significant potassium-solubilizing effects.

References

[1] ZHONG WH, CAI ZC. Effect of soil management practices and environmental factors on soil microbial diversity: A review[J]. Biodiversity Science, 2004(4): 456 – 465. (in Chinese).

[2] FAN XG, JIN KE, LI ZJ, *et al.* Soil microbial diversity under different fertilization and tillage practices: A review[J]. Journal of Plant Nutrition and Fertilizers, 2010, 16(3): 744 – 751. (in Chinese).

[3] REN HY, XU Q, QI XJ, *et al.* Differences of bacterial and fungal communities in the tree and rhizosphere of the healthy and twig blight-diseased bayberry[J]. Chinese Journal of Applied Ecology, 2021, 32(9): 3107 – 3118. (in Chinese).

[4] XIANG LG, WANG HC, GUO ZN, *et al.* The influence of black shank disease infection on fungal community structure of rhizosphere soil and stem of tobacco plants[J]. Mycosystema, 2019, 38(12): 2099 – 2111. (in Chinese).

[5] WANG AH, YIN YP, XIONG HL, *et al.* Endophytic bacterial diversity analysis of Huanglongbing pathogen-infected citrus phloem tissue in Guangxi[J]. Scientia Agricultura Sinica, 2010, 43(23): 4823 – 4833. (in Chinese).

[6] WANG F, YIN YP, SUN LQ, *et al.* Endophytic bacterial community in the symptoms and symptomless tissues of HLB-affected citrus plant[J]. Acta Microbiologica Sinica, 2014, 54(8): 868 – 875. (in Chinese).

[7] LIU B, ZHENG XF, SUN DG, *et al.* The community structure of endophytic bacteria in different parts of huanglongbing-affected citrus plants[J]. Acta Ecologica Sinica, 2011, 31(24): 7325 – 7342. (in Chinese).

[8] XIONG DW, JIN DF, GU BT, *et al.* Endophytic bacterial community in Gannan navel orange affected by Huanglongbing[J]. Microbiology China, 2017, 44(3): 631 – 638. (in Chinese).

[9] ZHANG SM. Isolation and characterization of antifungal lipopeptides produced by endophytic *Bacillus amyloliquefaciens* TF28[J]. African Journal of Microbiology Research, 2012, 6(8): 1747 – 1755.

[10] HU GP, ZHENG XF, YOU MS, *et al.* Recent advances in research on endophytes[J]. Fujian Journal of Agricultural Sciences, 2010, 25(2): 226 – 234. (in Chinese).

[11] XU HM. The control effeciency and the microbial flora analysis of minituber common scab biocontrol[D]. Baoding: Hebei Agricultural University, 2015. (in Chinese).

[12] LUO F. Diversity of culturable endophytic and inter-root microorganisms of Dongxiang wild rice and its plant-promoting vitality[D]. Nanchang: Jiangxi Normal University, 2011. (in Chinese).

[13] DONG XZ, CAI MY. Handbook of systematic identification of common bacteria [M]. Beijing: Science Publishing House, 2001. (in Chinese).

[14] ALTSCHUL SF, GISH W, MILLER W. Basic local alignment search tool[J]. Journal of Molecular Biology, 1990, 215(3): 403 – 410.

[15] WANG J. Screening and characterization of blueberry inter-root growth-promoting bacteria and their growth-promoting effects [D]. Tai'an: Shandong Agricultural University, 2016. (in Chinese).

[16] LIU LH, PENG GX, HUANG SF, *et al.* Diversity and growth promotion of endophytic diazotrophic bacteria isolated from *Bryophyllum pinnatum* [J]. Microbiology China, 2019, 46(10): 2538 – 2547. (in Chinese).

[17] YANG YN. Screening and medium optimization of PGPR from tomato rhizosphere[D]. Tai'an: Shandong Agricultural University, 2017. (in Chinese).

[18] CUI YL, LI YM, LI LJ, *et al.* Effect of cadmium in the V-Ti tailings soil on the potassium-dissolving ability of potassium-dissolving bacteria [J]. Chinese Journal of Applied and Environmental Biology, 2019, 25(1): 76 – 82. (in Chinese).

[19] WANG Y. Screening, medium optimization and genome sequencing of peony inter-root growth promoting bacteria[D]. Tai'an: Shandong Agricultural University, 2017. (in Chinese).

[20] MACHUCA A, PEREIRA G, AGUIAR A, *et al.* Metal-chelating compounds produced by ectomycorrhizal fungi collected from pine plantations [J]. Letters in Applied Microbiology, 2007, 44(1): 7 – 12.

[21] WANG H, HAN LZ. Identification of four plant growth-promoting rhizobacteria isolated from tea rhizosphere[J]. Microbiology China, 2019, 46(3): 548 – 562. (in Chinese).

[22] TAN K, WU ZY, GU YL, *et al.* Screening of a high potassium-releasing microbial strain [J]. Journal of Liupanshui Normal University, 2018, 30(3): 52 – 55. (in Chinese).

[23] SONG C, SONG SS, JIA ZH. Isolation, screening and identification of high efficient potassium resolving bacteria and its growth-promoting effects on cucumber in mountains[J]. Jiangsu Agricultural Sciences, 2020, 48(17): 266 – 270. (in Chinese).

[24] JAISINGH R, KUMAR A, DHIMAN M. Isolation and characterization of PGPR from rhizosphere of *Sesame indicum* L.[J]. International Journal of Advanced Research in Biological Sciences, 2016, 3(3): 238 – 244.

[25] YANG M. Isolation and screening of rhizosphere growth-promoting bacteria in pepper and disease resistance[D]. Shenyang: Shenyang Normal University, 2020. (in Chinese).