Biological Characteristics of the Pathogen *Phyllosticta capitalensis* Causing Banana Freckle Disease in Hainan Province

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Abstract [**Objectives**] This study was conducted to clarify the biological characteristics of the pathogen *Phyllosticta capitalensis*, the causal agent of freckle disease on Cavendish banana in Hainan Province, China. [**Methods**] The impact of various nutritional and environmental factors, including media, carbon sources, nitrogen sources, temperature, pH and light on the growth and sporulation of *P. capitalensis* was assessed using two distinct methods: mycelium growth rate and blood counting chamber. [**Results**] The mycelial growth and sporulation of *P. capitalensis* on different media exhibited notable differences. The use of banana leaf extract dextrose agar (BLEAD) and carrot agar (CA) was observed to facilitate rapid mycelial growth. The potato dextrose agar (PDA) and potato sucrose agar (PSA) were conducive to the production of conidia. The utilization of distinct carbon and nitrogen sources exerted a pronounced influence on the growth of *P. capitalensis*. Maltose, dextrose, fructose, and casein acid hydrolysate were the preferred substrates for mycelial growth. The tested carbon and nitrogen sources did not significantly stimulate conidial production, whereas dextrose and $NaNO_3$ were found to favor sporulation. The optimal temperature for mycelial growth and conidial production was determined to be 28 and 32 ℃, respectively. No mycelial growth was observed at 5 ℃. Active mycelial growth was observed at pH 6 - 10, with pH 6 - 7 being particularly conducive to sporulation. Complete darkness was conducive to mycelial growth and sporulation. [**Conclusions**] It is recommended that BLEDA and PDA should be incubated at 28 ℃ for 14 d in the dark for the purpose of mycelial growth and sporulation of *P. capitalensis*, respectively.

Key words Cavendish banana; Freckle disease; *Phyllosticta capitalensis*; Mycelial growth; Sporulation

1 Introduction

Banana (*Musa* spp.) is an important economic crop in tropical and subtropical regions worldwide. The prevalence of diseases is a significant determinant of both the yield and the quality of banana crops. Freckle disease caused by *Phyllosticta* spp. has become a significant economic threat to banana plantations in Southeast Asia, where the fruit is cultivated for export, because the disease negatively impacts the marketability of the fruit[1-3]. The species *P. musarum*, *P. musaechinensis*, and *P. cavendishii* have been reported in association with banana freckle disease in the Chinese banana plantation region[4-6]. A recent report has identified *P. capitalensis* as a casual agent of freckle disease in multiple regions of China, including Chongqing, Guizhou and Guangxi[5,7-8]. In 2024, the fungal pathogen *P. capitalensis* was initially isolated from the Cavendish banana in Hainan, China and subsequently subjected to a pathogenicity test. Its identity was then established through a combination of morphological and cultural characteristics, ITS and LSU sequence analyses[9]. As a recently identified etiological agent of banana freckle disease in Hainan, it is necessary to conduct a comprehensive investigation into its biological characteristics to establish a foundation for effective disease control strategies.

2 Materials and methods

2.1 Inoculum preparation In this study, the CATAS-PC01 isolate of *P. capitalensis* obtained from a banana plantation in Hainan, China was employed. The 14-day-old cultures were grown and incubated at 28 ℃ in the dark on potato dextrose agar (PDA: potato 200 g/L, dextrose 20 g/L, agar 18 g/L) and subsequently subjected to the following tests.

2.2 Effect of different media on mycelial growth and sporulation The evaluation of mycelial growth and sporulation were conducted on six media: potato dextrose agar (PDA), potato sucrose agar (PSA: potato 200 g/L, sucrose 20 g/L, agar 18 g/L), oatmeal agar (OA: oatmeal 30 g/L, agar 18 g/L), carrot agar (CA: carrot 200 g/L, agar 18 g/L), cornmeal agar (CMA: cornmeal 30 g/L, agar 18 g/L), and banana leaf extract dextrose agar (BLEDA: banana leaf 200 g/L, dextrose 20 g/L, agar 18 g/L). Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a Petri dish containing one of six media and incubated at 28 ℃ in the absence of light. After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.3 Effect of carbon source on mycelial growth and sporulation The Czapek agar (CZA) medium was composed of the following ingredients: $NaNO_3$ (3 g/L), K_2HPO_4 (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), KCl (0.5 g/L), $FeSO_4$ (0.01 g/L), sucrose (30 g/L), and agar (18 g/L), with a pH adjusted to 7.0. The CZA without the addition of sucrose was used as the base medium (CK). The following carbon sources were separately added to the base medium

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(30 g/L) to evaluate the effect of the carbon source on mycelial growth and sporulation: maltose, dextrose, lactose, sucrose, fructose, and sorbital. Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a plate containing a distinct carbon source and incubated at 28 ℃ in the absence of light. After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.4 Effect of nitrogen source on mycelial growth and sporulation The CZA without the addition of $NaNO_3$ was used as the base medium (CK). The following nitrogen sources were separately added in the base medium (3 g/L) to evaluate the effect of the nitrogen source on mycelial growth and sporulation: urea yeast power, $(NH_4)_2SO_4$, $NaNO_3$, peptone, and casein acid hydrolysate. Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a plate containing a distinct nitrogen source and incubated at 28 ℃ in the absence of light. After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.5 Effect of temperature on mycelium growth and sporulation Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a fresh PDA Petri dish and incubated in the dark at a series of temperatures (5, 10, 15, 20, 25, 28, 32, 37, 40 ℃) for 14 d, respectively. After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.6 Effect of pH on mycelial growth and sporulation In a biosafety cabinet, autoclaved PDA medium in seven flasks, which had been cooled to 60 ℃, was adjusted to pH 5, 6, 7, 8, 9 and 10 with 1 mol/L HCl and 1 mol/L NaOH, respectively. Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a fresh PDA Petri dish at varying pH values and incubated at 28 ℃ in the dark. After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.7 Effect of light on mycelial growth and sporulation Mycelial plugs (5 mm in diameter) were excised from the active edge of 14-day-old colonies of *P. capitalensis* cultivated on PDA. Each mycelial plug was transferred to the center of a fresh PDA Petri dish and incubated in the dark at 28 ℃ under the three different light conditions (full light, 12 h alternating light and dark, and full darkness). After 14 d, the colony morphologies were observed and photographed. The colony diameters were measured using the cross intersect method, and the number of spores was counted using a blood counting chamber.

2.8 Statistical analysis Each treatment was repeated three times. The data were subjected to a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. In all cases, the significant level was set at $P<0.05$.

3 Results and analysis

3.1 Effect of different media on mycelial growth and sporulation *P. capitalensis* demonstrated the capacity to grow on all six media types tested (Table 1). The growth rate was the greatest on BLEDA, with colony diameters reaching (71.42 ± 1.01) mm on day 14, a significant improvement over the other media ($P<0.05$). This growth pattern was followed by that observed on CA, CMA, and OA. The colony diameter on PDA was observed to be (27.67 ± 1.04) mm. The growth rate was the slowest on PSA, and the resulting colonies were the smallest and thinnest. The colony diameter was only (19.83 ± 0.76) mm. The media had a notable impact on the sporulation of *P. capitalensis* (Table 1). The highest spore yield (3.18×10^3 spores/mm^2) was observed on PDA, which exhibited a statistically significant difference compared to the other media ($P<0.05$). PSA demonstrated a relatively lower spore yield (1.17×10^3 spores/mm^2). The production of spores was unsuccessful on CMA, BLEDA, OA and CA.

Table 1 Effect of different media on the mycelial growth and sporulation of *Phyllosticta capitalensis*

Medium	Colony diameter//mm	Spore yield//10^3 spores/mm^2
CMA	51.00 ± 2.78 c	0 ± 0 c
BLEDA	71.42 ± 1.01 a	0 ± 0 c
OA	43.50 ± 0.50 d	0 ± 0 c
PDA	27.67 ± 1.04 e	3.18 ± 0.04 a
PSA	19.83 ± 0.76 f	1.17 ± 0.08 b
CA	67.33 ± 1.26 b	0 ± 0 c

NOTE Data in the table are expressed as mean ± standard deviation. Different lowercase letters in the same column indicate a statistically significant difference at the 0.05 level, as determined by Duncan's new multiple range test. The same below.

3.2 Effect of carbon source on mycelial growth and sporulation *P. capitalensis* demonstrated the capacity to grow on all six carbon nutrient sources (Table 2). The carbon sources maltose, dextrose, and fructose were found to be significantly more effective than the others ($P<0.05$). The colony diameters of the fungus on media were found to be (48.17 ± 3.83), (46.92 ± 2.74), and (41 ± 1.73) mm, respectively. Sucrose and lactose were the subsequent carbon sources, with colony diameters on media containing these carbon sources measuring (26.83 ± 2.25) and (19.08 ± 2.1) mm, respectively. The slowest growth was observed in the medium containing sorbital, with the colony diameter measuring (8.33 ± 1.04) mm. The sporulation of *P. capitalensis* was found to be significantly affected by the carbon source (Table 2). The highest spore yield (0.288×10^3 spores/mm^2) was observed

in media containing dextrose, which was significantly superior to the other carbon sources ($P<0.05$). Sucrose, maltose and lactose also supported spore yield, with 0.048×10^3, 0.032×10^3 and 0.016×10^3 spores/mm^2, respectively. The production of spores was not observed on media containing sorbitol and fructose.

Table 2 Effect of different carbon sources on the mycelial growth and sporulation of *Phyllosticta capitalensis*

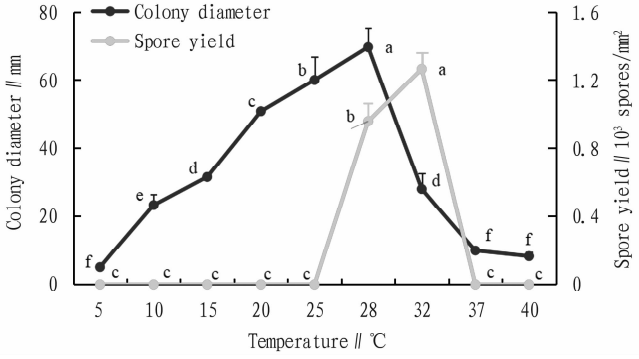
Carbon source	Colony diameter//mm	Spore yield//10^3 spores/mm^2
CK	8.83 ±0.72 e	0 ±0 d
Maltose	48.17 ±3.83 a	0.032 ±0.009 b
Dextrose	46.92 ±2.74 ab	0.288 ±0 a
Lactose	19.08 ±2.10 d	0.016 ±0 c
Sucrose	26.83 ±2.25 c	0.048 ±0.016 b
Fructose	41.00 ±1.73 b	0 ±0 d
Sorbitol	8.33 ±1.04 e	0 ±0 d

3.3 Effect of nitrogen source on mycelial growth and sporulation *P. capitalensis* demonstrated the capacity to grow on all six nitrogen nutrient sources (Table 3). The fastest growth was observed in the medium amended with casein acid hydrolysate. The colony diameter was observed to be (65.5 ±1.73) mm after incubation at 28 ℃ for 14 d, which was significantly superior to the results obtained with the other nitrogen sources ($P<0.05$). The colony diameters of *P. capitalensis* on media containing yeast power, peptone, $NaNO_3$ and $(NH_4)_2SO_4$ were found to be (33.33 ±1.36) mm, (32.58 ±0.88) mm, (32.35 ±1.47) mm and (30.08 ±1.38) mm, respectively. The fungus did not demonstrate growth on the medium containing urea. The nitrogen source had a marked impact on the sporulation of *P. capitalensis* (Table 3). The spore yield (0.016×10^3 spores/mm^2) was the highest on the medium containing $NaNO_3$, exhibiting a statistically significant improvement compared to the other nitrogen sources ($P<0.05$). The production of spores was not observed on media containing the other five nitrogen nutrient sources.

Table 3 Effect of different nitrogen sources on the mycelial growth and sporulation of *Phyllosticta capitalensis*

Nitrogen source	Colony diameter//mm	Spore yield 10^3 spores/mm^2
CK	33.17 ±0.80 b	0 ±0 b
Urea	5.00 ±0 c	0 ±0 b
Yeast power	33.33 ±1.36 b	0 ±0 b
$(NH_4)_2SO_4$	30.08 ±1.38 b	0 ±0 b
$NaNO_3$	32.25 ±1.47 b	0.016 ±0 a
Peptone	32.58 ±0.88 b	0 ±0 b
Casein acid hydrolysate	65.50 ±1.73 a	0 ±0 b

3.4 Effect of temperature on mycelium growth and sporulation *P. capitalensis* demonstrated the capacity to proliferate within a temperature range of 10 ℃ to 40 ℃ (Fig. 1). The optimal temperature was 28 ℃, and the colony diameter was (69.5 ± 5.77) mm, which was significantly superior to the other media ($P<0.05$), followed by 25, 20, 15, 32 and 10 ℃. The diameter exhibited no statistically significant difference at either 5 ℃ or 40 ℃ ($P>0.05$). No growth was observed at 5 ℃. The sporulation of *P. capitalensis* was found to be significantly affected by temperature (Fig. 1). The highest spore yield (1.26×10^3 spores/mm^2) was observed at a temperature of 32 ℃, which was significantly higher than the other temperature treatments ($P<0.05$). The second highest yield (0.96×10^3 spores/mm^2) was recorded at 28 ℃. The production of spores was not observed under any of the other seven temperature treatments.



NOTE Values are the means of three experimental runs, each with three replicates. Bar = standard deviation of mean. The same below.

Fig. 1 Effect of temperature on mycelial growth and sporulation of *Phyllosticta capitalensis* on PDA after 14 d of incubation

3.5 Effect of pH on mycelial growth and sporulation *P. capitalensis* demonstrated the capacity to grow at pH levels ranging from 4 to 10 (Fig. 2). No significant difference was observed in colony diameter between pH 6 and 9 ($P>0.05$). The optimal pH was 6, with a colony diameter of (82 ±0.5) mm. Subsequent pH values, 7, 8, 9, and 10, demonstrated progressively smaller colony diameters. At pH 4 and 5, a significant reduction in colony diameter was observed ($P<0.05$). The pH level exerted a notable influence on sporulation in *P. capitalensis* (Fig. 2). The highest spore yield (0.64×10^3 spores/mm^2) was observed at a pH of 7, which was significantly higher than the other temperature treatments ($P<0.05$). The production of spores was not observed at pH 4 and 5.

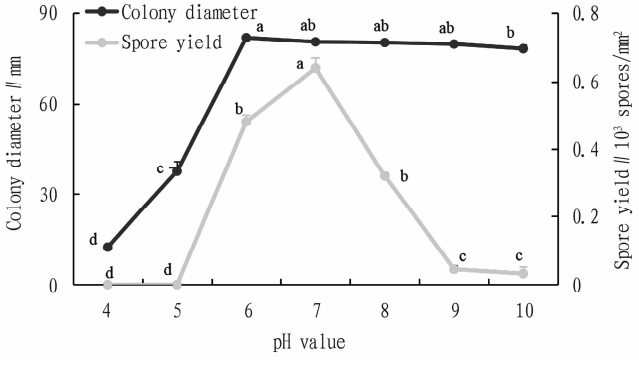


Fig. 2 Effect of temperature on mycelial growth and sporulation of *Phyllosticta capitalensis* on PDA after 14 d of incubation

3.6 Effect of light on mycelial growth and sporulation *P. capitalensis* demonstrated the capacity to grow under three different light conditions (Table 4). The fungus demonstrated optimal growth under light conditions of full darkness, exhibiting a colony

diameter of (57.17 ± 3.86) mm. This outcome was significantly superior to that observed under the other light treatments ($P < 0.05$). The 12 h alternating light and dark yielded a colony diameter of (37.83 ± 2.42) mm, while the full light treatment resulted in a colony diameter of (27.83 ± 2.76) mm. The sporulation of *P. capitalensis* was found to be significantly affected by light (Table 4). The spore yield (0.592×10^3 spores/mm^2) was the highest under the full darkness treatment, exhibiting a statistically significant improvement compared to the other light treatments ($P < 0.05$). The full light treatment (0.24×10^3 spores/mm^2) and the 12 h alternating light and dark treatment (0.112×10^3 spores/mm^2) demonstrated intermediated yields.

Table 4 Effect of different light on the mycelial growth and sporulation of *Phyllosticta capitalensis*

Light	Colony diameter//mm	Spore yield 10^3 spores/mm^2
Full light	27.83 ± 2.76 c	0.240 ± 0.01 b
12 h alternating light and dark	37.83 ± 2.42 b	0.112 ± 0.02 c
Full darkness	57.17 ± 3.86 a	0.592 ± 0.03 a

4 Discussion

The growth and sporulation of fungal mycelia were found to be significantly influenced by a number of factors, including the nutritional factors (medium, carbon source or nitrogen source) and environmental factors (temperature, pH or light). The mycelial growth of *P. capitalensis*, isolated from a banana planting area in Guangxi, China, was observed to be optimal in the presence of fructose and arabinose as carbon sources, and peptone and yeast extract as nitrogen sources, and the most active growth was observed at 28 ℃ with a pH of 6 and under full light[8]. This research results showed that the growth and sporulation of *P. capitalensis*, isolated from a banana planting area in Hainan, China, were significantly influenced by different media. The most appropriate media for mycelial growth were BLEDA and CA, while PDA and PSA were the most suitable for sporulation. The utilization of disparate carbon and nitrogen sources exerted a pronounced influence on the growth and sporulation of *P. capitalensis*. The carbon sources maltose, dextrose and fructose were found to be suitable for mycelial growth, with dextrose identified as the optimal carbon source for sporulation. The optimal nitrogen source for mycelial growth was casein acid hydrolysate, while $NaNO_3$ was the most effective for sporulation. The optimal temperature for mycelial growth was observed to be 25 – 28 ℃, with 28 ℃ being the most favorable temperature for this process and 32 ℃ being the most favorable temperature for sporulation. The pH range that facilitated optimal mycelial growth was 6 – 10, with pH 6 being the most favorable. For sporulation, the optimal pH was 7. A complete absence of light was conducive to the growth of mycelia and the formation of spores. The discrepancies in outcomes between the two studies may be attributed to the inherent variability in the response to environmental conditions among the isolates utilized in the studies.

In summary, in this study, we identified nutritional and environmental conditions conducive to the rapid growth and sporulation of the fungus *P. capitalensis*, isolated from a banana planting area in Hainan, China. The data yielded will prove invaluable for advancing our understanding of the fungus and the disease it causes through mycological and pathological research.

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