

VOLATILE ANTIFUNGAL COMPOUNDS PRODUCED BY PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AGAINST *Phytophthora palmivora*

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ABSTRACT – Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria that colonize plant roots and enhance plant growth by a diverse range of mechanisms and activate plant defense against microbial pathogens. In addition, volatile organic compounds (VOCs) are low-molecular-weight, carbon-containing compounds that evaporate easily at normal temperature and pressure. These compounds are biodegradable and can be as effective in disease control as conventional fungicides. In this study, a total of ten rhizospheric microbes were screened in vitro for their antagonistic effects against *Phytophthora palmivora*, the causal agent of Black Pod disease in cocoa. The effects of these rhizospheric microbes differed significantly in terms of their ability to inhibit the radial growth of *P. palmivora* after 168 hours of incubation using the dual culture method. After 168 hours of incubation, bacterial strain B2 were identified as the most promising antagonists against *P. palmivora*, achieving 58.71% inhibition of radial growth followed by B7 (57.59%) and B10 (56.10%). Further analysis of the volatile compound production potential of these three promising rhizospheric microbes revealed that volatile compound production was inversely proportional to the growth of *P. palmivora* and directly proportional to the percentage of inhibition.

Keywords: Volatile Compounds (VOCs), Plant Growth Promoting Rhizobacteria (PGPR), *Phytophthora palmivora*, dual culture method, black pod disease

INTRODUCTION

Contemporary agricultural research has increasingly focused on the multifaceted roles of plant-associated microorganisms in providing comprehensive protection against diverse environmental stresses, encompassing both biotic threats from pathogenic organisms and abiotic challenges arising from environmental fluctuations. Within the complex and dynamic soil microbial ecosystem, Plant Growth Promoting Rhizobacteria (PGPR) have emerged as the most prominent and influential beneficial microorganism, distinguished by their remarkable ability to establish sophisticated symbiotic relationships with plant root systems through intricate molecular communication networks (Mitra *et al.*, 2024).

These specialized rhizobacteria possess demonstrated efficacy in multiple aspects of plant health enhancement, including the promotion of robust plant developmental processes, significant improvement in nutrient acquisition efficiency from soil substrates, and substantial strengthening of plant defense

mechanisms against a wide spectrum of pathogenic organisms (Backer *et al.*, 2018). The multifaceted benefits provided by PGPR extend beyond direct plant growth promotion, encompassing complex biochemical interactions that fundamentally alter the rhizosphere environment.

One of the most distinctive and scientifically significant characteristics of PGPR is their remarkable capacity to synthesize and release diverse arrays of volatile organic compounds (VOCs) (Jishma *et al.*, 2017). These bioactive molecules, characterized by their relatively low molecular weight and inherent tendency to readily volatilize under standard ambient temperature conditions, represent a sophisticated chemical communication system within the soil ecosystem (Gouda *et al.*, 2018). The continuous biosynthesis and emission of VOCs by rhizobacteria populations contribute substantially to the structural complexity and functional diversity of the soil microbiome, thereby serving as key modulators of fundamental ecosystem processes including nutrient cycling dynamics, soil health maintenance, and microbial community stability (Baloch *et al.*, 2024).

The biological control of plant disease represents a sophisticated ecological phenomenon encompassing multiple interconnected and synergistic mechanisms. These complex processes include competitive exclusion strategies for limited nutrients and available ecological niches, strategic secretion of specific extracellular enzymes capable of degrading pathogen structures, and the targeted biosynthesis of potent antimicrobial compounds including both antibiotics and volatile organic compounds. Within these intricate biological control frameworks, VOCs have garnered scientific attention due to their unique properties as low-molecular-weight organic compounds characterized by high vapor pressure and broad-spectrum antimicrobial activity. These volatile compounds have progressively emerged as crucial and highly effective biocontrol agents in the sustainable management of various plant pathogens, offering environmentally friendly alternatives to conventional chemical pesticides while maintaining high efficacy in pathogen suppression and plant protection (Ling *et al.*, 2022).

Of agricultural significance is the application of PGPR-derived VOCs in controlling *P. palmivora*, a devastating hemibiotrophic oomycete pathogen that represents one of the most economically important threats to cocoa (*Theobroma cacao*. L) production worldwide (Thomas *et al.*, 2011). *P. palmivora* is capable of infecting host tissue through specialized infection structure including germ tubes, appressoria, and haustoria, which facilitate both adherence and nutrient extraction during its hemibiotrophic colonization and infected over 200 plants species and causes significant damage to cocoa plants, particularly through black pod disease, resulting in approximately 20-30% of global crop loss (Perrine-Walker, 2020) and can lead to up to 10% tree mortality each year (Guest, 2007).

The application of PGPR VOCs against *P. palmivora* represents a promising biocontrol strategy that operates through multiple inhibitory mechanisms. Studies on in vitro assays have shown that endophytic bacterial isolates from cocoa rhizosphere and root tissues particularly *Pseudomonas aeruginosa* AS1 and *Chryseobacterium proteolyticum* AS2 can inhibit *P. palmivora* radial growth by over 80%, with volatilome profiling highlighting VOCs as key antagonistic agents (Alsultan *et al.*, 2019). Research has verified the presence of Phytophthora species, suggesting that VOCs can disrupt critical developmental processes essential for pathogen establishment and proliferation (Syed-Ab-Rahman *et al.*, 2019)

The effectiveness of PGPR VOCs in retarding *P. palmivora* growth extends beyond direct antimicrobial activity, encompassing interference with zoospore motility, disruption of appressorium formation, and inhibition of houstonium development. These multifac-

eted inhibitory effects collectively contribute to the reduction of disease incidence and severity, offering sustainable protection for cocoa crops against this economically devastating pathogen while promoting overall plant health and productivity (Penaud *et al.*, 2025). Therefore, this study was carried out to evaluate the potential of PGPR to exhibit antagonistic effects against *P. palmivora* in the laboratory at the Cocoa Research and Development Centre, Jengka, Pahang.

MATERIALS AND METHODS

This study was conducted at the Cocoa Research and Development Centre (CRDC), Jengka, Pahang. Root samples were collected from three locations within the CRDC field plot. The samples were obtained from healthy, mature cocoa trees (20-25 years old) with yields exceeding 0.5 tonnes per hectare and no signs of Vascular Streak Dieback or Black Pod disease. Root segments (10-15 cm) were excavated from the intact root system at a distance of 45-60 cm from the tree base using a surface-sterilized hoe. From these samples, a total of ten rhizosphere microbes were isolated and subsequently screened in vitro for their antagonistic activity using the dual culture method as described by Rajani *et al.* (2021).

Culture disc (5mm diameter) of both *P. palmivora* and the PGPR isolates were excised from the margins of seven-day old active cultures using a sterile cork borer. These discs were then placed on PDA medium contained in petri plates (90mm diameter) on opposite sides approximately 10mm from the edge of the plate, while rhizosphere bacterial isolates were streaked on the opposite side of the petri plate. A control plate containing only the test pathogen was prepared for comparison.

All plate was wrapped with parafilm and incubated at room temperature (29°C-33°C) under 12 h of light/dark cycles. A total of 132 plates were prepared for this experiment, corresponding to 11 experimental treatments, corresponding to 11 experimental treatments (10) rhizospheric microbes and 1 control) with 12 replications per treatment. The experimental treatments were arranged in a Completely Randomized Design (CRD). Antagonistic effects were recorded every 24 hours for a total of 168 hours by measuring the radial growth of *P. palmivora*. The percentage of growth inhibition was then calculating using the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

Data concerning mycelial inhibition were subjected to Analysis of Variance (ANOVA). The means obtained were subsequently compared using the

Duncan Multiple Range Test (DMRT) at a significant level of $p \leq 0.05$. All statistical analyses were performed using the RStudio (Version 2024.12.1 Build 563).

RESULTS AND DISCUSSIONS

The antagonistic effects of ten rhizosphere isolates against *P. palmivora* were evaluated through volatile organic compound (VOCs) production in vitro. A one-way Analysis of Variance (ANOVA) confirmed that there were significant differences ($p < 0.05$) among the treatments.

The complete data and statistical details are presented in Table 1. Based on the post-hoc test, the isolates were categorized into three main performance groups. The highest performing group consisted of isolates B2 (58.71%), B7 (57.59%), and B10 (56.10%). This was followed by an intermediate group of five isolates showing moderate inhibition. The lowest performing group consisted of isolates B9 (49.22%) and B3 (49.03%), which were significantly less effective than the highest performing group. Critically, all ten isolates were significantly more effective at inhibiting *P. palmivora* growth compared to the control. Our findings align with previous research by Pengnoo *et al.*, (2025), who documented that native rhizospheric microbes isolated from soil can effectively inhibit *P. palmivora* growth.

Table 1: Percentage of mycelial growth inhibition of *P. palmivora* by VOCs from ten rhizospheric bacterial isolates.

No.	Bacterial isolates	Mean inhibition rate (%)
1.	B2	58.71a
2.	B7	57.59ab
3.	B10	56.10ab
4.	B4	55.17abc
5.	B6	54.80abc
6.	B1	53.87abc
7.	B8	52.75abc
8.	B5	51.45bc
9.	B9	49.22c
10.	B3	49.03c
11.	Control	0.00d

*Means in the same column followed by the same letter are not significantly different based on DMRT at $p < 0.05$. SED (Standard Error of difference) = 0.104; CD (Critical Difference) = 0.21.

CONCLUSIONS

This study highlights the significant potential of native plant growth promoting rhizobacteria (PGPR) as biological control agents against *P. palmivora*, the pathogen responsible for black pod disease. All tested PGPR

isolates demonstrated antagonistic activity in vitro, with ten isolates inhibiting mycelial growth by 49.70% to 58.71%. These findings align with previous research on rhizosphere microbes and provide further evidence that PGPR can serve as effective alternatives to chemical fungicides. The antagonistic properties of these microbes are likely related to their ability to efficiently colonize the rhizosphere and produce antifungal secondary metabolites. Overall, this research offers valuable insights into sustainable disease management strategies that can reduce reliance on chemical fungicides and promote environmentally friendly cocoa production.

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REFERENCES

- Alsultan, W., Vadamalai, G., Khairulmazmi, A, Saud, H.M., Al-Sadi, A. M., Rashed, O., Jaaffar, A.K. & Nasehi, A. (2019). Isolation, identification and characterization of endophytic bacteria antagonistic to *Phytophthora palmivora* causing black pod of cocoa in Malaysia. *European Journal of Plant Pathology*, 155(4), 1077-1091)
- Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramaniam, S. & Smith, D.L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in plant science*, 9, 1473.
- Baloch, F.B., Zeng, N., Gong, H., Zhang, Z., Zhang, N., Baloch, S.B., Ali, S. & Li, B. (2024). Rhizobacterial volatile organic compounds: Implications for agricultural ecosystems nutrient cycling and soil health. *Heliyon*, 10 (23).
- Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S. & Patra, J.K. (2018). Revitalization of plant growth promoting rhizobacteria for

- sustainable development in agriculture. *Microbiological research*, 206, 131-140.
- Guest, D. (2007). Black pod: diverse pathogens with a global impact on cocoa yield. *Phytopathology*, 97(12), 1650-1653.
- Jishma, P., Hussain, N., Chellapan, R., Rajendran, R., Matthew, J. & Radhakrishnan, R.K. (2017). Starin-specific variation in plant growth promoting volatile organic compounds production by five different *Pseudomonas* spp. as confirmed by response of *Vigna radiata* seedlings. *Journal of Applied Microbiology*, 123, 204-216.
- Ling, L., Luo, H., Yang, C., Wang, Y., Cheng, W., Pang, M. & Jiang, K. (2022). Volatile organic compounds produced by *Bacillus velezensis* L1 as a potential biocontrol agent against postharvest diseases of wolfberry. *Frontiers in Microbiology*, 13, 987844.
- Mitra, D., Pellegrini, M. & Guerra-Sierra, B.E. (2024). Interaction between plants and growth-promoting rhizobacteria (PGPR) for sustainable development. *Bacteria*, 3(3), 136-140.
- Penaud, V., Alahmad, A., De Vrieze, M., Bouteiller, M., Eude, M., Bernardon-Mery, A., Trinsoutrot-Gattin, I., Lava, K. & Gauthier, A. (2025). In vitro biocontrol potential of plant extract-based formulation against infection structures of *Phytophthora infestans* along with lower non-target effects. *Frontiers in Microbiology*, 16, 1569281.
- Pengnoo, A., Lohlaeh, U., Maduerehand, F., Kaewmaho, C., Krualae, S., Wongpisal, P., Homhaul, W., Boonyapipat, P., Saeng-ngam, S., Akbarjan, A. & Phuntumart, V. (2025). *Bacillus velezensis* SM 1: A promising biocontrol solution for *Phytophthora* durian root rot. *Applied microbiology*, 5 (1),21.
- Perrine-Walker, F. (2020). *Phytophthora palmivora* – cocoa interaction. *Journal of fungi*, 6(3), 167.
- Rajani, P., Rajasekaran, C., Vasanthakumari, M. M., Olsson, S. B., Ravikanth, G. & Shaanker, R. u. (2021). Inhibition of plant pathogenic fungi by endophytic *Trichoderma* spp. through mycoparasitism and volatile organic compounds. *Microbiological Research*, 242, 126595
- Syed-Ab- Rahman, S.F., Carvalhais, L.C., Chua, E.T., Chung, F.Y., Moyle, P.M., Eltanahy, E.G. & Schenk, P.M. (2019). Soil bacterial diffusible and volatile organic compounds inhibit *Phytophthora capsica* and promote plant growth. *Science of the Total Environment*, 692, 267-280.
- Thomas, L., Gupta, A., Gopal, M., Chandra Mohanan, R., George, P. & Thomas, G.V. (2011). Evaluation of rhizospheric and endophytic *Bacillus* spp. and fluorescent *Pseudomonas* spp. isolated from *Theobroma cacao* L. for antagonistic reaction to *Phytophthora palmivora*, the causal organisms of black pod disease of cocoa. *Journals of plantation crops*, 39 (3): 370-376.