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ORIGINAL ARTICLE

Understanding colonization and proliferation potential of endophytes and pathogen *in planta* via plating, polymerase chain reaction, and ergosterol assay

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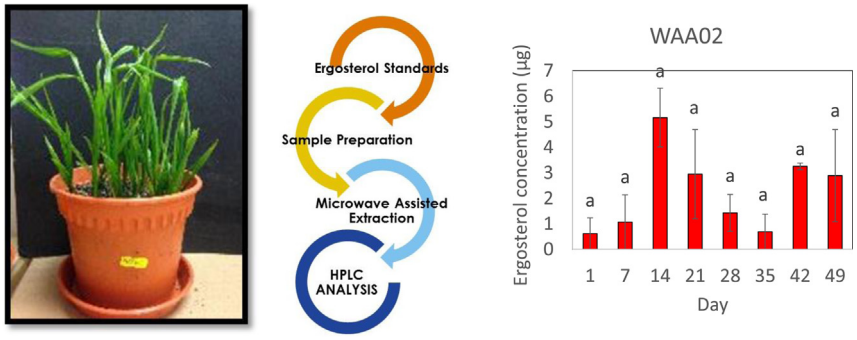
GRAPHICAL ABSTRACT



Colonization and proliferation potential of endophytes and pathogen *in planta* via ergosterol assay and compared to conventional plating and PCR methods.

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ABSTRACT

This study aimed to establish the colonization behavior and proliferation potential of three endophytes and one pathogen *Ganoderma boninense* (Gb) introduced into oil palm ramets (host model). The endophytes selected were *Diaporthe phaseolorum* (WAA02), *Trichoderma asperellum* (T2), and *Penicillium citrinum* (BTF08). Ramets were first inoculated with 100 mL of fungal cells (10^6 cfu mL^{-1}) via soil drenching. For the next 7 days, ramets were sampled and subjected to three different assays to detect and identify fungal colonization, and establish their proliferation potential *in planta*. Plate assay revealed the presence of endophytes in root, stem and leaf tissues within 7 days after inoculation. Polymerase Chain Reaction (PCR) detected and identified the isolates from the plant tissues. The ergosterol assay (via high-performance liquid chromatography, HPLC) confirmed the presence of endophytes and Gb *in planta*. The increase in ergosterol levels throughout 49 days was however insignificant, suggesting that proliferation may be absent or may occur very slowly *in planta*. This study strongly suggests that the selected endophytes could colonize the host upon inoculation, but proliferation occurs at a slower rate, which may subsequently influence the biocontrol expression of endophytes against the pathogen.

Introduction

Endophytes are microorganisms that reside inside the internal tissues of living plants without causing any symptoms to the host plants [1,2]. They can be found in various plants growing in the tropics, temperate regions and in boreal forests [3]. Endophytes are valuable as they produce a variety of bioactive compounds [4]. They are also known to have biocontrol potential against several important plant pathogens [5], either by inducing plant defense mechanisms [6] or by promoting plant growth [7]. The presence of endophytic biocontrol agents (BCAs) in the plants is advantageous as endophytes are protected from adverse soil conditions [8,9]. Several studies have reported the successful use of endophytic BCAs, mainly on vegetable and fruit crops. Chinese cabbage seedlings treated with the endophyte *Heteroconium chaetospira* were resistant to the pathogen *Plasmodiophora brassicae* [1]. Endophytes were also able to protect tomatoes [10], banana [11], barley and beans [12], against their respective pathogens. In addition, the presence of endophytes also improved plant growth. Improved vegetative growth was observed in maize, tobacco and parsley treated with endophytic *Pirifomospora indica* [13], as well as pigeon-peas and bananas treated with non-pathogenic *Fusarium* isolates [11,14]. Improved plant growth leads to robust plants which are less susceptible to pathogen infection. Endophytic BCAs have also been tested on oil palm to control *Ganoderma boninense* (Gb) and these include endophytic bacteria *Burkholderia cepacia* and *Pseudomonas aeruginosa* [5] and species of the mycoparasitic *Trichoderma* sp. [15–17].

Application of endophytic BCAs was however, less effective than chemicals in controlling diseases [18]. Several factors contribute to this, with nonconductive soil conditions (abiotic and biotic factors) as the primary cause of concern. Soil factors are hypothesized to have inhibited the growth of BCAs, leading to poor (or absence of) disease control by BCAs [19]. It was further explained that the survival of introduced BCAs may have been impeded by the intense competition by indigenous microbiota in the soil, or by the poor physicochemical soil conditions [20,21]. In this study, we propose that the colonization behavior and proliferation potential of endophytes *in planta* may be a contributing factor influencing their subsequent biocontrol activity. The ability of endophytes to colonize plant tissues successfully is essential for controlling plant diseases and providing benefits to plants. Their ability to proliferate indicates how readily endophytes are able to adapt and grow inside the plants. This hypothesis is novel, and suggests that the manner endophytes colonize, grow and proliferate in host tissues is important to their subsequent effectiveness as BCAs. The colonization and proliferation potential of endophytes *in planta* could be determinative factors that subsequently impact disease suppression.

To test this hypothesis, the colonization and proliferation potential of endophytes was compared to the oil palm pathogen (*Ganoderma boninense*, Gb) and studied using a model host plant (oil palm). The endophytes (WAA02, T2, BTF08) selected were known BCAs that are antagonistic toward Gb [22] and *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4) [11]. The colonization and proliferation potential of endophytes was compared to Gb as both endophytes and pathogen compete for similar niche. Gb is a pathogen rampant in the tropics, but the infection and colonization of oil palm by Gb are poorly understood [23]. Gb is known to be able to colonize young oil palm tissues, but only cause disease symptoms at a later growth stage, suggesting that Gb remained a successful colonizer of host tissues for a relatively long period of time [24,25]. In this study, assessments were carried out using three approaches. Plating and PCR were first conducted to demonstrate that endophytes and pathogen are able to enter into plant tissues. PCR further identified the correct species of endophyte (and pathogen) present in the tissues. Growth of endophytes and pathogen was then assessed via ergosterol quantification assay. Ergosterol assay was adopted in this study as it has been widely used as an indicator to estimate fungal biomass in various environments such as air [26], food [27,28], leaf litter [29], mycorrhizal roots [30] and soil [31].