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Characterizing antagonistic activities and host compatibility (via simple endophyte-calli test) of endophytes as biocontrol agents of *Ganoderma boninense*

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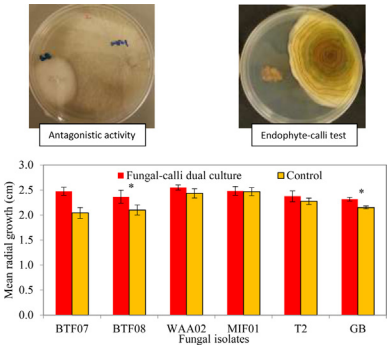
HIGHLIGHTS

- Endophytes inhibited pathogen via inhibitory compounds and competitive exclusion.
- BTF08 is compatible with host-plant with growth promoting effects towards calli.
- Calli produced substances that stimulated growth of BTF08 and GB.
- Endophyte-calli assay results are validated by endophyte-ramet test.
- *P. citrinum* BTF08 and *T. asperellum* T2 have most potential as biocontrol agents.

GRAPHICAL ABSTRACT



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ABSTRACT

This study characterized the antagonistic activities of five fungal endophytes (*Aspergillus calidoustous* BTF07, *Penicillium citrinum* BTF08, *Trichoderma asperellum* T2, *Diaporthe phaseolorum* WAA02, *Diaporthe phaseolorum* MIF01) and evaluated their endophyte-host compatibility with the host plant (oil palm). The antifungal activities of the endophytes towards *Ganoderma boninense* (GB) were first established using the dual culture test, revealing antagonistic nature of endophytes via production of non-volatiles, volatiles and competitive exclusion. Endophyte-host compatibility was then assessed using a simple but rapid endophyte-calli dual-culture assay, and results validated using endophyte-ramet test. Results revealed that endophytes elicited different responses in oil palm calli. BTF08 had growth promoting effects towards the host tissues with the highest calli weight (1013 mg) obtained, while BTF07 appeared to inhibit calli (1006 mg) leading to browning and necrosis. This endophyte-calli test also revealed the influence of calli on endophyte growth. Isolates BTF08 and GB benefited from host association, with increased radial growth (2.36 cm and 2.31 cm, respectively) compared to growth in the absence of calli (2.10 cm and 2.15 cm, respectively). Endophytes and GB were also isolated from host tissues, suggesting compatibility and ability to colonize host tissues (root, stem, leaf). This suggested the reliability of the endophyte-calli test as a rapid assay to provide an insight on the endophyte-host compatibility.

1. Introduction

Endophytes are microorganisms which colonize the internal plant tissues without causing any disease symptoms (Arnold,

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2007). In recent years, endophytes have been studied for various purposes such as for their plant growth regulating properties, increased tolerance towards environmental changes, antagonistic properties towards pathogens and in triggering induced-host resistance in plants (Arnold, 2007; Duijff et al., 1997; Pleban et al., 1995; Porras-Alfaro and Bayman, 2011; Sturz et al., 1998). Several of these applications (e.g. biological control, tolerance to abiotic stress) require the introduction of endophytes into "new" host plants (Postma and Rattink, 1992; Thomas and Upreti, 2014). Under these circumstances, endophytes are isolated from one host plant and purposefully inoculated into a different/new host plant to produce the desired effect. The association and interaction between the introduced endophyte with the "new" host plant will gradually determine their symbiotic response (mutualistic, antagonistic or synergistic). It is hypothesized that the interaction between endophytes and host could either lead to subsequent proliferation or inhibition of the endophytes in the host plant. This ultimately influences their expression of beneficial characteristics when present in the host plants.

It is therefore paramount that the compatibility between endophyte and the host is investigated, particularly for antagonistic endophytes with biocontrol potential. Several profound findings were reported, which include the following; (i) endophyte colonization in plants are achieved by artificial inoculation of conidia, ascospores and mycelium through wounding of the plant tissues (Lahrmann et al., 2013; Leuchtmann, 1993); (ii) colonization frequency varies in different parts (leaf, stem, root and flower) (Masumi et al. (2015); (iii) and possible tissue-specificity traits in certain endophyte genera such as the detection of *Stemphylium* and *Aspergillus* in leaf tissues, *Ulocladium*, *Drechslera* and *Curvularia* from stem tissues, and *Cylindrocarpon* from root tissues of *Thymus* sp., respectively (Masumi et al. (2015). The endophyte-host compatibility is also said to be influenced by chemical recognition between host and fungi (Ride, 1992; Tyler, 2002). Chapela et al. (1991) reported that endophytic *Xylariaceous* species responds to certain chemical compounds in the host plants such as monolignol glucosides, which influences ascospore germination. Thus, the function of chemical molecules produced by host tissues is observed to have a certain degree of influence on the growth and proliferation of endophytes in the host tissues. It is also hypothesized by Dupont et al. (2015) and Eaton et al. (2010) that introduced endophytes could elicit recognition mechanisms in the new host plant to either accept or reject endophyte colonization.

To date, none of the studies were on commercially important agronomic crops such as oil palm as studies on endophytes and oil palm mostly focused on endophyte-growth promoting effects (Anuar et al., 2015) and biocontrol (Sundram et al., 2015), not on the endophyte-host compatibility. Hence, the endophytes tested were potential biocontrol agents of *Ganoderma boninense*, a pathogen causing devastating loss to the oil palm industry (Rajesh et al., 2014). These endophytic isolates (*Aspergillus calidoustous* BTF07, *Penicillium citrinum* BTF08, *Trichoderma asperellum* T2, *Diaporthe phaseolorum* WAA02, *Diaporthe phaseolorum* MIF01) were selected as they belong to genera with known antifungal properties (producers of inhibitory compounds, mycoparasitic action, competitive-exclusion) (Akrami et al., 2011; Ting et al., 2009a,b, 2012, 2010; Yu et al., 2010). In addition to antagonistic properties, it is highly desirable that these endophytes also have good compatibility with the host plant. This study was therefore conducted to define the compatibility of the endophytes with oil palm using a rapid assay; the endophyte-calli assay.

The endophyte-calli assay is a simple endophyte-host model based on the *in vitro* dual culture concept, using endophyte and oil palm calli co-inoculation for rapid observation of their interactions. This model used the calli of oil palm because a calli cell mimics the actively-dividing cells of the cambium tissues of a plant (Hendry et al., 1993; Nawrot-Chorabik, 2013). This report is the first to present the combination of calli-test model and colonization studies as useful assays to determine endophyte-host compatibility.

2. Materials and methods

2.1. Culture establishment

Fungal endophytes were first isolated from various host plants; isolate BTF07, BTF08 and T2 from stem tissues of *Musa* sp. (Ting et al., 2009b, 2012; Ting and Jioe, 2016), isolate WAA02 from roots of *Portulaca* sp. (Ting et al., 2009b, 2010), and isolate MIF01 (Ting et al., 2010) from roots of *Mimosa pudica*. The endophytes were identified as *Aspergillus calidoustous* BTF07 (GenBank accession No. KT329189), *Penicillium citrinum* BTF08 (GenBank accession No. KT964566), *Trichoderma asperellum* T2 (GenBank accession No. KT964564) and two isolates of *Diaporthe phaseolorum* MIF01 (GenBank accession No. KT964565) and WAA02 (GenBank accession No. KT964567). These isolates demonstrated good biocontrol potential; with *T. asperellum* (T2), *P. citrinum* (BTF08), *D. phaseolorum* (WAA02) showing complete overgrowth towards *Ganoderma boninense* under metal-stress (Ting and Jioe, 2016). Other researchers have also identified the biocontrol activities of these isolates against another pathogen (*Fusarium oxysporum*), establishing the antifungal potential of the selected endophytes (Akrami et al., 2011; Ting et al., 2009a,b, 2012; Yu et al., 2010). The pathogen, *G. boninense* was obtained from Prof. Dr. Sariah Meon, Universiti Putra Malaysia. All fungal cultures (endophytes and pathogen) were maintained on Potato Dextrose Agar (PDA) (Merck) at room temperature (25 ± 2 °C).

2.2. In vitro screening for antagonistic activity

In vitro screening for antagonistic activity of the selected endophytes against *G. boninense* was evaluated by dual culture assay. Antagonistic activity of endophytes towards *G. boninense* were evaluated through dual culture test (Albert et al., 2011; Meon, 1998). Control plates were established by co-inoculating *G. boninense* with PDA agar plug. All tests were conducted with three replicates and incubated at room temperature. The diameter of *G. boninense* was recorded. The inhibition percentage was recorded after incubating for a week. Inhibition of *G. boninense* by endophyte was calculated based on Percentage of Inhibition of Radial Growth (PIRG):

$$\text{PIRG}\ (\%) = \frac{R1 - R2}{R1} \times 100\%$$

where R1: radial growth of *G. boninense* co-inoculated with plain agar plug (control), and R2: radial growth of *G. boninense* co-inoculated with fungal endophytes.

2.3. Calli-endophyte dual culture test

The dual culture test was adopted from Peters et al. (1998), where petri dishes were first filled with 25 ml callus multiplication medium (Murashige and Skoog medium supplemented with 2 mg 2,4-dichlorophenoxyacetic acid, 5 mg 6-Benzylaminopurine, and 7 g phytoagar) (Murashige and Skoog, 1962). In our study, we used two-week old oil palm calli (supplied by Applied Agricultural Resources Sdn. Bhd.) and they were transferred onto the agar. Mycelial plug of 7-day old endophyte culture was then co-inoculated onto the agar at an equi-distance of 2 cm from the periphery of the plates. For observation of calli growth without the influence of endophytes, plain agar plugs (instead of endophyte