available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/funeco

A variety of melanised root-associated fungi from the Sydney basin form endophytic associations with *Trifolium subterraneum*

T.T. MUKASA MUGERWA*, J.A. SALEEBA, P.A. MCGEE

A12, School of Biological Sciences, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 4 June 2012
Accepted 26 July 2012
Available online 18 October 2012
Corresponding editor:
Kevin Hyde

Keywords:
Ascomycota
Dark septate endophytes
Endophyte
Melanin
Mutualism
Zygomycota

ABSTRACT

Knowledge of the abundance, diversity, and plant interactions of melanised root-associated fungi remains limited. The objective of this study was to isolate a wide variety of melanised root-associated fungi within the Sydney basin (NSW, Australia) and assess growth response of *Trifolium subterraneum* to inoculation with individual isolates. Of 902 root-associated fungi isolated from plant roots, 118 were melanised. All but two of these fungi were re-isolated from inoculated *T. subterraneum* seedlings after 7 weeks in a controlled environment. Approximately 60 % of the melanised root-associated fungi did not reduce plant growth. Twenty-four isolates tended to increase plant growth and were tentatively identified as predominantly ascomycetes, and one zygomycete. Melanised root-associated fungi appeared to form complex interactions with *T. subterraneum*, the natures of which remain to be further explored. Melanised root-associated fungi could potentially play key ecological roles including positively influencing edaphic conditions.

Introduction

The interactions between roots and soil fungi range from mutualism to antagonism (Bonfante & Genre 2010). The most common interaction involves arbuscular mycorrhizal fungi which form mutualistic associations with most terrestrial vascular plants (Smith & Read 2008; Smith & Smith 2012). Arbuscular mycorrhizal fungi are obligate biotrophs that colonise roots and derive their nutrients from host plants. The arbuscular mycorrhizal fungus–plant association is mutually beneficial: arbuscular mycorrhizal fungi enhance acquisition of minerals from the surrounding soil (Doubková *et al.* 2012; Karasawa *et al.* 2012; Smith & Smith 2012). Pathogens can also utilise the plant as their primary source of nutrients, though some pathogenic fungi have both a root-colonising and a saprotrophic phase (Termorshuizen & Jeger 2008). Plants and their metabolic products can thus influence the growth, activity and distribution of fungi in soil (Wardle *et al.* 2004).

A wide variety of fungi can be isolated from plant tissues (Gazis *et al.* 2011; Purahong & Hyde 2011; Sánchez Márquez *et al.* 2012; Suryanarayanan *et al.* 2012). Interactions between these fungi and the host plant range, and the mechanisms of these interactions are often unclear (Rodriguez *et al.* 2009; Saikkonen *et al.* 2010; Torres *et al.* 2012). Endophytic fungi colonise living plant tissues for part or all of their life cycle, without apparent detrimental effects on their host (Hyde & Soytong 2008; Linnakoski *et al.* 2012). Similar to arbuscular mycorrhizal fungi (Smith & Smith 2012), endophytes may

* *Corresponding author*. Tel.: +61 2 9351 4675; fax: +61 2 9351 4771.
E-mail address: t.mukasamugerwa@sydney.edu.au (T.T. Mukasa Mugerwa).
1754-5048/$ – see front matter
http://dx.doi.org/10.1016/j.funeco.2012.08.004

benefit through the provision of habitat or direct supply of nutrition from plants (Mack & Rudgers 2008). Endophytes are ubiquitous and are well documented in the above-ground tissues (shoots) of most plants (Faeth & Fagan 2002; de Errasti *et al*. 2010; Selvanathan *et al*. 2011; White & Bacon 2012). Shoot endophytes influence plants in a variety of ways, such as increasing their resistance to herbivores, and are therefore ecologically important in some cases (Rodriguez *et al*. 2009; Porras-Alfaro & Bayman 2011; White & Bacon 2012).

Far less is known about non-mycorrhizal and non-pathogenic root-associated endophytic fungi (Vaz *et al*. 2011). Interest in a widely encountered group of melanised root-associated fungi designated as dark septate endophytes has recently increased (Alberton *et al*. 2010; Newsham 2011). Dark septate endophytes are widespread, taxonomically diverse conidial or sterile ascomycetes that may form melanised structures such as inter- and intracellular hyphae and microsclerotia in roots (Barrow 2003; Mandyam & Jumpponen 2005). The host range of dark septate endophytes includes plant species that do not form arbuscular mycorrhizal associations (Jumpponen & Trappe 1998a; Mandyam & Jumpponen 2005; Usuki & Narisawa 2007). Dark septate endophytes and arbuscular mycorrhizal fungi, however, can interact in roots (Menoyo *et al*. 2007; Scervino *et al*. 2009). Dark septate endophytes may stimulate plant growth under specific environmental conditions (Jumpponen *et al*. 1998; Jumpponen 2001; Mandyam *et al*. 2012). The enzymatic capabilities of some dark septate endophytes further indicate their potential to access soil-borne sources of organic nitrogen and phosphorus (Mandyam *et al*. 2010). Like arbuscular mycorrhizal fungi, dark septate endophytes may influence the plant-fungus interaction by modifying the surrounding soil.

Some functions of dark septate endophytes may also be related to the presence of melanin. Melanins are complex biopolymers synthesised from phenolic and aromatic compounds. The compounds are expressed by a wide range of fungi (Suryanarayanan *et al*. 2004; Dong & Yao 2012). While not required for the primary growth and development of fungi, melanins may enable fungi to tolerate various environmental stresses as well as enhance their virulence in pathogenic interactions (Butler & Day 1998; Eisenman & Casadevall 2012). Melanin may potentially be held in soil for longer periods than other organic compounds and thus play a significant role in the carbon cycle. Direct and indirect plant and ecosystem function might, thus, be attributable to melanised root-associated fungi.

As melanised root-associated fungi form associations with plants that range from pathogenic to mutualistic (Mandyam *et al*. 2012), we predict that a subset may directly or indirectly benefit their host plant, as has been found for arbuscular mycorrhizal fungi (Smith & Smith 2012). Alternatively, subsets of melanised root-associated fungi may either have no effect or even retard plant growth. Melanised root-associated fungi may have either transient or long-lived associations with roots and the interaction may change with environment and/or over time. The precise role of each root-associated fungus may differ and be difficult to resolve.

This study is the first step of a larger project to investigate the potential for melanised root-associated fungi to influence edaphic conditions. The objectives of this study were to:

1. Indicate the prevalence and range of melanised root-associated fungi within the Sydney basin (NSW, Australia).
2. Determine the patterns of interaction between melanised root-associated fungi and *Trifolium subterraneum*.
3. Indicate whether melanised root-associated fungi can influence soil conditions.

Materials and methods

Collection of root material

Roots were collected from a variety of plant species within remnant native vegetation across a range of locations within and around the city of Sydney (NSW, Australia) between Mar. and Aug. 2009 (Supplementary Table 1). Collection sites were selected to incorporate northern, southern, western and eastern (central) localities of the Sydney basin (NSW, Australia). Each site had differing plant communities, soil types and local environments (Fairley & Moore 2000) with potentially differing root-associated fungal communities. The mean daily maximum temperature in Sydney is approximately 21.5 °C and the mean annual rainfall is 1220 mm (Bureau of Meteorology 2009, Observatory Hill, Sydney, NSW, Australia). As many root systems of plants were intermingled, roots were collected without attempting to identify the host plant. Approximately 5–10 g of apparently healthy root material from single plants of a variety of native plants at each location was extracted from soil using a trowel and placed in an airtight zip-lock bag (GLAD, Snap Lock) to prevent desiccation. Samples were transported at ambient temperature to the School of Biological Sciences, The University of Sydney (Camperdown Campus, Sydney, NSW, Australia) and stored at 5 °C until further analysis.

Isolation of melanised root-associated fungi

Within 48 hr of collection, excess soil was shaken from the roots and then the roots thoroughly washed with reverse osmosis (RO) water before surface sterilisation by submersion in 70 % ethanol for 10 s, followed by submersion in sodium hypochlorite (2 % chlorine) for 2 min in a laminar flow work station (GELMAN Sciences). Samples were removed aseptically from the hypochlorite solution and washed three times with autoclaved (Pacs 2000, GETINGE) (121 °C for 15 min) RO water.

Ten root fragments (1 cm length) from each freshly washed root system were aseptically sectioned and each fragment placed onto Potato Dextrose Agar containing 12.5 mg l^{-1} streptomycin and tetracycline (PDA) (Difco) within sterilised disposable plastic Petri dishes (Sarstedt). A maximum of five fragments were placed onto PDA within each Petri dish. The root fragments were incubated in the dark at 26 °C for 14 d (Cao *et al*. 2004). Hyphae emerging from root segments after the incubation period were recorded as root-associated fungi. Imprints of the freshly washed root systems were also made on separate PDA Petri dishes. Imprinted PDA Petri dishes were incubated in the dark at 26 °C for 7 d to ascertain whether surface sterilisation eliminated epiphytes.