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The dark septate endophytic fungus *Phialocephala fortinii* is a potential decomposer of soil organic compounds and a promoter of *Asparagus officinalis* growth

Surono [a, b, c], Kazuhiko Narisawa [a, b, *]

[a] United Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3-8-1 Harumi-cho, Fuchu-shi, Tokyo 183-8538, Japan
[b] College of Agriculture, Ibaraki University, 3–21–1 Chuo, Ami, Ibaraki 300–0393, Japan
[c] Indonesian Agency for Agricultural Research and Development, Jl. Ragunan No.29, Pasar Minggu, Jakarta 12540, Indonesia

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ABSTRACT

There is limited information as to whether dark septate endophytic (DSE) fungi are able to degrade organic carbon, nitrogen and phosphorus compounds in soil and if these fungi have a significant role in nutrient cycles in nature, especially under organic nutrient conditions. In order to further knowledge in this area, 25 DSE fungi were isolated and tested for their promotion of *Asparagus officinalis* seedling growth. Three *Phialocephala fortinii* isolates were found to be most effective in increasing the growth of *A. officinalis* plants compared with uninoculated controls. These isolates had the ability to degrade all carbon and nitrogen compounds tested except for lignin. Using organic phosphorus and nitrogen sources, the three *P. fortinii* isolates were able to promote the growth of *A. officinalis* compared with control plants. The isolates were also able to promote the growth of *A. officinalis* seedlings on semi-organic and organic media. Our findings demonstrate that *P. fortinii* has a role in the promotion of *A. officinalis* growth under organic nutrient conditions, possibly by decomposing organic phosphorus and nitrogen compounds in soil.

1. Introduction

More than 90% of all terrestrial plant species enter into symbiotic relationships either with mycorrhizal or endophytic fungi, including dark septate endophytic (DSE) fungi, in natural ecosystems (Carroll, 1988; Shahollari et al., 2007; Behie et al., 2013). Recently, DSE fungi have been shown to be abundant in natural forest ecosystems and have shown promise for application in agricultural systems, although their positive impact on plants in natural ecosystems needs to be studied further (Hennon et al., 1990; Addy et al., 2000; Mahmoud and Narisawa, 2013).

DSE are a group of endophytic fungi that generally have melanized hyphae, form dark colonies on agar media, and are able to colonize plant roots both inter- and intracellularly without causing disease symptoms (Jumpponen and Trappe, 1998; Thormann et al., 1999; Jumpponen, 2001; Wilson et al., 2004; Diene et al., 2013; Knapp et al., 2015). The role of DSE fungi in nature has been considered to be similar to that of mycorrhizal fungi (Peterson et al., 2008; Della Monica et al., 2015), although the mechanisms involved in nutrient transfer from DSE fungi to their hosts are still unclear. The well-known DSE fungus *Phialocephala fortinii* is able to enhance nitrogen (N) and phosphorus (P) uptake by pine. Although this phenomenon is amongst the better-known beneficial effects of DSE fungi (Smith and Read, 1997; Jumpponen and Trappe, 1998), more research is still needed to support the view that the fungus has mycorrhizal-like interactions with its host plants. *P. fortinii* is abundant in nature and generally has symbiotic relationships with several coniferous trees such as *Pinus sylvestris, Pinus contorta, Picea abies* and *Abies alba* (Konnert and Bergmann, 1995; Ahlich-Schlegel, 1997). It has not yet been determined if *P. fortinii* can be isolated from other coniferous trees dominant in natural Japanese forests, such as *Chamaecyparis obtusa* and *Cryptomeria japonica*.

P. fortinii is abundant in natural forest ecosystems in conditions rich with organic matter. Therefore, along with other fungal decomposers, it may have a significant role in organic material

* Corresponding author. Present address: Department of Bioresource Science, College of Agriculture, Ibaraki University, 3–21–1 Chuo, Ami, Ibaraki, 300–0393, Japan.
E-mail address: kazuhiko.narisawa.kkm@vc.ibaraki.ac.jp (K. Narisawa).

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decomposition in forest soils (Stoyke et al., 1992; Thormann et al., 1999; Menkis et al., 2005; Grünig et al., 2006). Caldwell et al. (2000) demonstrated that *P. fortinii* has the ability to degrade polymeric forms of carbon (C), N, and P such as cellulose, starch and protein. To support plant growth under organic conditions, it is important for DSE fungi such as *P. fortinii* to be able to degrade organic compounds to produce nutrients in forms available to its host plant. Information on the ability of DSE fungi to degrade organic polymeric forms of C, N and P is still limited, especially in relation to the use of organic media for promoting host plant growth. Recently, five DSE fungi, including *P. fortinii,* have been reported to decompose organic compounds (Caldwell et al., 2000; Menkis et al., 2004; Mandyam et al., 2010). Therefore, like ectomycorrhizal and ericoid mycorrhizal fungi, *P. fortinii* may have the ability to retrieve N and P from plant litter, although research in this area remains limited (Caldwell et al., 1996; Jumpponen and Trappe, 1998; Lindahl and Tunlid, 2015).

P. fortinii has been reported to be a plant growth promoter in many studies (Jumpponen et al., 1998; Jumpponen and Trappe, 1998; Narisawa et al., 2002, 2004; Vohník et al., 2005). However, some studies have found that *P. fortinii* has neutral or even negative effects on host plants (Wilcox and Wang, 1987; O'Dell et al., 1993; Fernando and Currah, 1996; Jumpponen and Trappe, 1998; Jumpponen, 2001; Vohník et al., 2005; Grünig et al., 2008). Clearly, the effect of DSE fungi on host plants, either positive or negative, still needs to be elucidated (Grünig et al., 2008). The role of *P. fortinii* in promoting plant growth under organic conditions and its preferences for organic N and P sources have yet to be reported. To date, only the DSE species *Heteroconium chaetospira, Pseudosigmoidea ibarakiensis* and *Scolecobasidium humicola* have been reported to promote plant growth under organic conditions when amino acids such as leucine and valine are used as organic N sources (Usuki and Narisawa, 2007; Diene et al., 2013; Mahmoud and Narisawa, 2013). Therefore, further *in vitro* studies are needed to select DSE that have positive effects on host plants before these fungi are applied in the field under organic conditions.

In this study, we focused on *Asparagus officinalis* as a target host plant, because of the species' increasing importance in agriculture (Zenkteler et al., 2012). *A. officinalis* is a perennial crop that can be cultivated for over 10 years and can be harvested continuously (Wang et al., 2010). Moreover, in *A. officinalis* cropping systems, a supply of organic fertilizer is needed to increase *A. officinalis* production (Espejo et al., 1997; Monokrousos et al., 2008; Xu et al., 2014). Therefore, the use of DSE fungi that can function either as plant growth promoters or as organic compound decomposers would be beneficial in such systems. Based on a previous report, *P. fortinii,* which may have the potential to be used to promote the growth of asparagus, can associate with *A. officinalis* (Yu et al., 2001). There are only a few reports relating to the association between DSE fungi and *A. officinalis*, in contrast to numerous reports of the associations between the plant species and arbuscular mycorrhizal fungi. *A. officinalis* plants are usually symbiotic with mycorrhizal fungi such as *Glomus* spp. and *Ambispora granatensis* (Burrows et al., 1990; Mizonobe et al., 1991; Palenzuela et al., 2011; Matsubara et al., 2014), which increase the absorption efficiency of P from soil (Xu et al., 2014).

The objectives of this study were to test (1) the ability of DSE fungi to promote *A. officinalis* growth, (2) the ability of DSE fungi to degrade C and N compounds, (3) the effect of DSE fungi on *A. officinalis* growth under organic N and P conditions, and (4) the effect of DSE fungi on *A. officinalis* growth under semi-organic and organic conditions.

2. Materials and methods

2.1. Isolation of dark septate endophytic fungi from plant roots in a natural environment

2.1.1. Plant materials

Plant root sampling was carried out in May 2015. We collected 580 root samples of Japanese cypress (*C. obtusa*), 80 of wild Japanese raspberry (*Rubus* sp.), 40 of skervish (*Erigeron philadelphicus*), 80 of wild strawberry (*Fragaria iinumae*), 104 of Japanese cedar (*C. japonica*), 70 of dandelion (*Taraxacum officinale*), 40 of phragmites (*Phragmites japonicus*), 42 of goldenrod (*Solidago* sp.), 40 of Japanese ginger (*Zingiber mioga*), 112 of wheat (*Triticum* sp.), and 40 of unidentified plant species. The 11 sites sampled were natural ecosystems close to the town of Ami (35°59′53″–36°04′29″ N, 140°12′17″–140°23′19″ E) and the cities of Tsuchiura (36°03′26″ N, 140°13′19″ E) and Kasumigaura (36°15′18″ N, 140°23′70″ E) in Ibaraki Prefecture, Japan.

2.1.2. Surface disinfection and fungal isolation

The root samples were washed with tap water to remove debris and then cut into approximately 10 mm segments. The segment root samples were washed three times using 0.005% solutions of Tween 20 or polyoxyethylene (20) sorbitan monolaurate (Wako, Pure Chemical Industries, Ltd., Japan), followed by three rinses in sterilized distilled water using a vortex mixer (CM-1000; Tokyo Rikakikai, Tokyo, Japan). Root segments, air-dried overnight, were then plated into 50% corn meal agar medium (Table 1) in 90 mm plastic Petri dishes (with three segments in each dish). For the purposes of identification, single fungal colonies were grown at 23 °C on 50% corn meal malt yeast medium in 60 mm Petri dishes (Table 1).

2.2. Dark septate endophyte fungal screening

2.2.1. Fungal isolates

DSE in this study were considered to be fungal isolates that were dark and slow-growing (growth rate < 3 mm per day), usually starting development after at least 7–14 days of incubation. All DSE fungal candidates isolated from roots collected from the natural environment (25 fungal isolates) were grown on oatmeal agar medium (Table 1) in 55 mm diameter Petri dishes. The fungi were incubated for three weeks at room temperature (approximately 23 °C).

2.2.2. Selection of isolates

A. officinalis seeds were surface sterilized by immersion in 70% ethanol for 1.5 min and then a solution of sodium hypochlorite (1% available chlorine) for 3 min. The seeds were rinsed three times with

Table 1
Composition of media used for DSE fungal isolation.

Constituent	50% Corn meal agar	50% Corn meal malt yeast agar	Oatmeal agar	2% Malt extract agar
Corn meal agar	8.5 g	8.5 g	0	0
Bacto agar	7.5 g	7.5 g	0	15 g
Malt extract	0	10 g	0	20 g
Yeast extract	0	1.0 g	0	0
Oatmeal	0	0	10 g	0
Agar powder	0	0	15 g	0
$MgSO_4 \cdot 7H_2O$	0	0	1.0 g	0
KH_2PO_4	0	0	1.5 g	0
$NaNO_3$	0	0	1.0 g	0
Distilled water	1000 mL	1000 mL	1000 mL	1000 mL