The cellulolytic activity and symbiotic potential of dark septate endophytic fungus \textit{Phialocephala fortinii} to promote non-mycorrhizal plants growth

Surono\textsuperscript{1} and K Narisawa\textsuperscript{2}

\textsuperscript{1}Indonesian Soil Research Institute, Bogor, Indonesia  
\textsuperscript{2}Graduate School of Agriculture, Ibaraki University, Ami-machi, Ibaraki, Japan

E-mail: Surono54@pertanian.go.id

Abstract. The endophytic fungi have attracted much attention to investigating their ability to degrade cellulose and promote plant growth as double functional roles. The objective of this study was to investigate the cellulase activity and the ability of dark septate endophytic fungus (DSE) \textit{Phialocephala fortinii} to promote non-mycorrhizal plants. This study consisted of carboxymethylcellulose degradation activity of DSE isolates, cellulase activity, and the effect of cellulolytic DSE to promote non-mycorrhizal plants growth assays. The results showed \textit{P. fortinii} III.Pi.18 had the largest cellulolytic index than \textit{P. fortinii} CKG.II.10.1 and \textit{P. eryngii} for 7 days of incubation time. The cellulase activity pattern of all \textit{P. fortinii} isolates was rather similar to \textit{P. eryngii} for the incubation time of 7 to 35 days. \textit{Phialocephala fortinii} III.Pi.18, CKG.II.10.1, and CKG.I.11 increased the growth of \textit{Brassica oleracea} var. \textit{capitata} by 7.63, 92.5, and 47.7\%, and \textit{Spinacia oleracea} by 58.5, 283, and 303\%, respectively, over to the control plant. Heavy colonization of \textit{P. fortinii} occurred both in \textit{B. oleracea} var. \textit{capitata} and \textit{S. oleracea} roots. This study proved that although \textit{P. fortinii} has cellulase activity but did not cause disease symptoms in non-mycorrhizal plants so that it can potentially be multifunctional as cellulose decomposer and plant growth promoter.

1. Introduction

Cellulose is one of the main components of plant cell walls [1] and the most abundant in the world as biopolymer [2]. It can be degraded by a cellulase enzyme that consisted of endoglucanase (endo-1,4-\(\beta\)-D-glucanase, EC 3.2.1.4), exo-glucanase (1,4-\(\beta\)-D-glucan-cellobiohydrolase, EC 3.2.1.91) and \(\beta\)-glucosidase (\(\beta\)-D-glucoside glucanohydrolase, cellobiase, EC 3.2.1.21), which act synergistically to degrade cellulose [3]. Various kinds of microbes including bacteria, fungi, and actinomycetes are involved in cellulose decomposition, but fungi are generally regarded as the main responsible organism for this process [4,5]. Fungi have the important role in nutrient cycling and promoting bioconversion of cellulose in nature [6,5]. Most of the cellulolytic fungi are saprophytic and parasitic [7]. Recently, the endophytic fungi have attracted much attention to investigating their ability to degrade cellulose and to promote plant growth without causing disease symptoms as double functional roles [8-10]. One of these endophytic fungal groups is dark septate endophytic fungi (DSE).

DSE fungi are the group of endophytes that most of which are Ascomycotina, form dark colonies on agar media and colonize plant root inter- and intracellularly without causing apparent negative effects, although several of DSE fungi have the ability to produce plant cell wall degrading enzymes.
such as cellulase and pectinase [11-13]. Many aspects of their ecological roles remain unclear although several studies had focused on the abundance and diversity of DSE in various ecosystems. Not only the diversity of DSE fungi are poorly understood but only a few of these taxa have been metabolically characterized such in enzymatic activity [14,12]. However, until now only five DSE species including P. fortinii that have been reported to degrade the main component of plant cell walls such as cellulose, starch and pectin [14-16].

We reported that three P. fortinii isolates gained from natural forest ecosystems in Japan were qualitatively capable of degrading cellulose in vitro [16] and P. fortinii isolates will be tested in the cellulolytic capability quantitatively by determining their cellulase activity in the current study. In addition, the P. fortinii isolates have been shown to promote the growth of Asparagus officinalis [16, 17], whereas in this study P. fortinii isolates will be inoculated into non-mycorrhizal plants to prove whether the isolates were able to build the symbiosis relationship with non-mycorrhizal plants or not. Only 18 of the vascular plants are not able to form an arbuscular mycorrhizal symbiosis [18]. Cabbage (Brassica oleracea var. capitata) and spinach (Spinacia oleracea) are used as non-mycorrhizal plants to be tested with P. fortinii because they are reportedly not symbiotic with mycorrhiza but symbiotic with other endophytic fungi [19,20]. Thus, the objective of this study was to investigate the cellulase activity of P. fortinii isolates and the ability to promote non-mycorrhizal plants.

2. Materials and methods

2.1. Dark septate endophytic and white rot fungal materials

The dark septate endophytic fungal isolates used in the current study were three DSE fungal isolates that promote A. officinalis from previous studies that consisting of P. fortinii isolates III.Pi.I8, CKG.I.11, and CKG.II.10.1 [16]. Basidiomycetous fungus Pleurotus eryngii, known as a white-rot fungus, was used as a control of cellulose decomposer fungus. All DSE fungal and P. eryngii isolates were available from Prof. Kazuhiro Narisawa, Laboratory of Microbial Ecology, Ibaraki University, Japan. All DSE fungal and cellulose-degrading fungal isolates were grown in Potato Dextrose Agar (PDA) media at a subsequent experiment.

2.2. Carboxymethyl cellulose degradation

CMC (Carboxymethylcellulose) agar and liquid media were used to test for the presence of fungal cellulase activity. Carboxymethyl cellulose (CMC) agar basal medium containing 200 mL CMC (as the cellulose source, 10 g CMC in 200 mL distilled water), 1 g KH$_2$PO$_4$, 0.5 g K$_2$SO$_4$, 0.5 g NaCl, 0.5 g FeSO$_4$, 1 g NH$_4$NO$_3$, 0.01 g MnSO$_4$, 20 g agar and 1 L distilled water was used to determine cellulose degradation activity [21,16].

2.3. Cellulase activity assay using High-Performance Liquid Chromatography (HPLC)

All DSE fungi and P. eryngii were grown in CMC broth media with composition like previous treatment and sugar reducing was determined every 7 days. For measuring cellulolytic activity referred to methods by Schwald et al. [22] and Chu et al. [23]. Sugar reducing in cultures was determined by HPLC (JASCO, Tokyo, Japan) equipped with Shodex SUGAR SH1011 column (8.0 mm ID by 300 mm) under the following condition: 25°C column temperature; 5 mM H$_2$SO$_4$ mobile phase; 0.60 ml min$^{-1}$ flow rate.

2.4. Effect of cellulolytic dark septate endophytic fungi to promote non-mycorrhizal plants growth

The procedures of these treatments referred to Surono and Narisawa [16] in the screening test of DSE fungi to promote plant growth, but different in the host plants. Cabbage (B. oleracea var. capitata) cv. Teruyoshi (Nippon Norin Seed, Japan) and Spinach (S. oleracea) cv. Misaki (Sakata Seed, Japan) were used as non-mycorrhizal plant hosts.
2.5. Anatomical observation
To observe the effects of selected DSE fungi on the roots of *B. oleracea* var. *capitata* and *S. oleracea*, 3-week-old *B. oleracea* var. *capitata* and *S. oleracea* seedling roots were washed, cross-sectioned used Microtome (Leica Biosystems Nussloch GmbH, Germany), and stained with 50% acetic acid solution containing 0.005% cotton blue, before being examined using a light microscope (BX51; Olympus, Tokyo, Japan).

2.6. Data analysis
The mean of cellulase index, cellulase activity, and dry biomass of each treatment were calculated and analyzed using one-way ANOVA with R version 3.0.2 (The R Foundation for Statistical Computing Platform, Vienna, Austria). Differences among treatment means were detected using Tukey’s Honestly Significant Difference test (Tukey HSD).

3. Results and discussion

3.1. Cellulolytic index of selected *Phialocephala fortinii*
This study showed that *P. fortinii* have ability to degrade cellulose as well as *P. eryngii*. *Phialocephala fortinii* isolate III.Pi.I8 grew more slowly than *P. fortinii* isolates CKG.I.11 and CKG.II.10.1 in the cellulose agar medium. However, *P. fortinii* isolate III.Pi.I8 had largest cellulolytic index than *P. fortinii* isolates CKG.II.10.1 and *P. eryngii* for 7 days incubation time using (table 1). Therefore, they have potential to degrade cellulose.

The capability of cellulose degradation may be one of *P. fortinii*’s important roles in nature where the fungus lives abundantly in the natural forest ecosystem usually with rich organic matter content, although this phenomenon has not yet been investigated further [24, 25, 26, 27, 28]. In soil ecosystem, the role of decomposer fungi is very important being capable of breaking down complex organic compounds into simple forms that microbes and plants can use to promote their growth [29]. *Phialocephala fortinii* have a unique potential both as plant growth promoter [25,31-33,16] as well as decomposer of organic materials, especially cellulose although the adequate dual function of the research has not realized.

| Isolate          | Diameter of colony (mm) | Diameter of cellulolytic zone (mm) | Cellulolytic index |
|------------------|-------------------------|------------------------------------|-------------------|
| III.Pi.I8        | 9.91<sup>a</sup>        | 28.2<sup>b</sup>                   | 1.85<sup>c</sup>  |
| CKG.I.11         | 13.1<sup>b</sup>        | 36.9<sup>c</sup>                   | 1.81<sup>c</sup>  |
| CKG.II.10.1      | 10.1<sup>a</sup>        | 22.7<sup>a</sup>                   | 1.26<sup>b</sup>  |
| *P. eryngii*     | 26.0<sup>c</sup>        | 26.8<sup>b</sup>                   | 1.03<sup>a</sup>  |
| CV (%)           | 6.55                    | 4.49                               | 9.37              |

<sup>a</sup>Values within columns followed by the same letter are not significantly different (P<0.05) after Tukey’s Honestly Significant Difference test.

3.2. Cellulase activity of selected *Phialocephala fortinii* compared with *Pleurotus eryngii*
The cellulase activity of *P. fortinii* isolate CKG.I.11 was highest compared with *P. fortinii* III.Pi.I8 and CKG.II.10.1 in 7 days of incubation time. However, if compared with *Pleurotus eryngii*, cellulase activity of these *P. fortinii* isolates was lower in 7 days of incubation time. As well as in 7, 14, 21, 28 and 35 days of incubation time, the cellulase activities of *P. eryngii* were higher than all *P. fortinii* isolates. The pattern of cellulase activity of *P. eryngii*, *P. fortinii* isolates CKGI.11 and CKGII.10.1
was similar. Their cellulase activity increased in 14, 28 and 35 days of incubation time, but it decreased in 21 days of incubation time. Whereas, cellulase activity of *P. fortinii* isolate III.Pi.18 increased in 14 and 35 days of incubation time and it decreased in 21 and 28 days of incubation time.

In the incubation time of 7-14 days, the cellulase activity of all *P. fortinii* isolates and *P. eryngii* increased, because the fungi may be requiring more glucose by degrading cellulose from the growth media to carry out their energy source [34], this pattern is almost similar among all *P. fortinii* isolates, as well as *P. eryngii*. While in the incubation time of 14-21 days, the cellulase activity of all fungi declined which may be due to the source of glucose that required by the fungi to be used in their growth activity was in a sufficient condition so that the production of cellulase enzyme declined. The increasing of cellulase activity was occurred again at the incubation time of 21-35 days on all tested fungi in this study. *Phialocephala fortinii* produced cellulase enzyme during the degradation of cellulose do that this organic substrate becomes a simple unit and can be utilized by these fungi and plants [35]. According to Deswal et al. [36] that cellulose degradation process by cellulase enzyme produced by microbes including *P. fortinii* is a time-consuming process and the treatment with different microbes also has different decomposition time [37]. However, the cellulase enzymes derived from fungi and bacteria are more stable than other sources [38].

![Cellulase activity by selected DSE fungi and *P. eryngii* in 35 days of incubation time](image)

**Figure 1.** Cellulase activity by selected DSE fungi and *P. eryngii* in 35 days of incubation time.

3.3. **Evaluation of the ability of cellulolytic dark septate endophytic fungi to promote non-mycorrhizal plants**

All isolates *P. fortinii* promoted *B. oleracea var. capitata* growth in this study without causing any typical disease symptoms. *Phialocephala fortinii* isolates III.Pi.18, CKG.II.10.1 and CKG.I.11 increased the growth of *B. oleracea var. capitata* by 7.63%, 92.5%, and 47.7%, respectively over to the control plant. However, *P. fortinii* isolates III.Pi.18 and CKG.I.11 were not different in promoting *B. oleracea var. capitata* growth compared with the control plant, while *P. fortinii* isolate CKG.II.10.2 was significant different to promote *B. oleracea var. capitata* growth compared with the control treatment. The performances of *B. oleracea var. capitata* associated with all *P. fortinii* isolates were better than control plant.
Figure 2. The effect of dark septate endophytic fungi to *B. oleracea var. capitata* growth on oatmeal agar media incubated at 23°C with a 16-h:8-h (L: D) photoperiod (180 mol m⁻² s⁻¹) for 3 weeks. Data are the mean±SD, n=3. Columns with the same letter are not significantly different (P<0.05) using Tukey’s Honestly Significant Difference test. CV = 23.4%.

All tested isolates *P. fortinii* promoted *S. oleracea* growth in this study without causing any disease symptoms. *Phialocephala fortinii* isolates III.Pi.I8, CKG.II.10.1 and CKG.I.11 increased the growth of *S. oleracea* by 58.5, 283 and 303%, respectively over to the control plant. The dry weight of *S. oleracea* inoculated with *P. fortinii* isolates CKG.I.1.11 and CKG.II.10.1 were significant different compared with the control plant. While dry weight of *S. oleracea* inoculated with *P. fortinii* isolate III.Pi.I8, although the increasing of the dry weight of this treatment was higher than the control plant.

Figure 3. The effect of dark septate endophytic fungi to *S. oleracea* growth on oatmeal agar media incubated at 23°C with a 16-h:8-h (L: D) photoperiod (180 mol m⁻² s⁻¹) for 3 weeks. Data are the mean±SD, n=3. Columns with the same letter are not significantly different (P<0.05) using Tukey’s Honestly Significant Difference test. CV = 28.2%.
Non-mycorrhizal plants such as species of Brassicaceae, Chenopodiaceae, Caryophyllaceae, Proteaceae, and Amaranthaceae are plants that are not associated with mycorrhiza in the field but may associate with other endophytic fungi including DSE fungi [39, 20]. The presence of AM fungi may inhibit the growth of non-mycorrhizal plants [40-42]. Several reports stated that some DSE fungi could associate with non-mycorrhizal plants. Heteroconium chaetospira [43] and Pseudosigmoidea ibarakiensis [44] associated with non-mycorrhizal plants without causing any disease symptoms. However, information about the association of P. fortinii with non-mycorrhizal plants such as Chinese cabbage, cabbage, lettuce, sugar beet and spinach has not been widely reported. In our study, all P. fortinii associated with non-mycorrhizal plants, B. oleracea var. capitata (Brassicaceae), and S. oleracea. P. fortinii isolate CKG.II.10.1 increased the highest growth of B. oleracea var. capitata compared to other P. fortinii isolates and control plants. Heavy colonization occurred in the roots of B. oleracea var. capitata by those fungal hyphae. This indicated that P. fortinii associated with non-mycorrhizal plant, B. oleracea var. capitata without reducing the cabbage dry weight. Similarly, when tested with the other non-mycorrhizal plant, S. oleracea, P. fortinii CKG.II.10.1 and CKG.I.11 significantly increased the growth of S. oleracea compared with S. oleracea associated with P. fortinii isolate III.PI.18 and the control plant. While P. fortinii III.PI.18 did not differ significantly in increasing the growth of both B. oleracea var. capitata and S. oleracea compared with the control treatment. It is likely that different isolates of P. fortinii differ in the ability to promote non-mycorrhizal plant growth. Non-mycorrhizal plants inoculated with P. fortinii could grow well without the appearance of disease symptoms either in root or shoot of plants. Although in the previous test, the three isolates were quantitatively capable of producing cellulase enzymes but did not cause any damages on living non-mycorrhizal plant cellulose. Both parasite and endophytic fungi penetrate the plant roots by producing cell wall degrading enzymes such as cellulase [45, 46]. However, the enzymatic activity of the endophytic fungus penetrates the cell wall of the plant and establish inside the roots does not cause any damages on/in the root tissue [47]. Most of P. fortinii are isolated from healthy plant tissues [48-51], as well as P. fortinii used in this study were isolated from healthy Japanese yam and raspberry roots, so that they potentially don't act as fungal parasites, although several reports stated that P. fortinii also act as weak parasites [52-54].

3.4. Anatomical observation
Heavy colonization occurred both in B. oleracea var. capitata and S. oleracea roots inoculated with P. fortinii isolate CKG.II.10.1 and CKG.I.11 especially within the epidermal and cortical cells (figure 4). However, the colonization pattern did not reach the vesicular systems both in B. oleracea var. capitata and S. oleracea roots. Penetration done by P. fortinii on the roots of B. oleracea var. capitata and S. oleracea did not cause damage to the roots although this fungus could produce cellulase enzyme that is one of the degrading plant cell walls enzymes. This proves that in general the species of DSE fungi could associate symbiotically with non-mycorrhizal plants based on reports of several previous studies such as H. chaetosphira [32], Veronaeopsis complex [55], and P. ibarakiensis [44] and at the present study using P. fortinii. However, Kastini et al. [55] reported that V. complex couldn't degrade cellulose in vitro [56] and no report regarding the cellulose degrading activity of H. chaetosphira and P. ibarakiensis, so that it cannot be explained yet whether the colonization mechanism in non-mycorrhizal roots with these DSE fungi is different or not with colonization performed by P. fortinii that could produce cellulase enzymes. Therefore, further research is needed to investigate the process or mechanism of colonization in non-mycorrhizal plant roots between species of DSE fungi that produce cellulase enzymes with DSE fungi that do not produce cellulase enzymes and their effect on the promotion of non-mycorrhizal plant growth.
Figure 4. Images of *P. fortinii* colonization in *B. oleracea* var. capitata roots (A- *P. fortinii* CKG.II.10.1, C- *P. fortinii* CKG.II.11) and *S. oleracea* roots (B- *P. fortinii* CKG.II.10.1, D- *P. fortinii* CKG.II.11). DSE fungal colonization can be seen on the root surface (arrows), within epidermal cells (Ep) and the cortex (Co). Note the absence of colonization in the vascular cylinder (Vc). Bars = 20 μm.

4. Conclusions
From the results of this study it can be concluded as follows that DSE fungus *P. fortinii* can degrade cellulose and its cellulose activity does not cause any negative effects on non-mycorrhizal plants as it is proven that these plants are promoted in growth so that the range of host plants is very wide and can be used in symbiosis with various plants. The DSE fungus *P. fortinii* has the potential to be multifunctional beneficial endophytic fungus so that it can be used as a cellulose decomposer and non-mycorrhizal plant growth promoter effectively.

Acknowledgments
This work was partly supported by Japan Society for The Promotion of Science (JSPS) KAKENHI Grant Number 17H03948 (to KN). The funding source had no role in study design; in the collection analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. S and KN designed the research analyzed and interpreted the data. S performed the experiments, provided data, and wrote the manuscript. All authors reviewed and edited the final manuscript.
References

[1] Kubicek C P 2013 Fungi and lignocellulosic biomass (New York: John Wiley and Sons)
[2] Bayer E A and Lamed R 1992 The cellulose paradox: pollutant par excellence and/or a reclaimable natural resource Biodegradation 3 171-188
[3] Sadhu S and Maiti T K 2013 Cellulase production by bacteria: a review British Microbiol. Res. J. 3(3) 235-258
[4] Khalid M, Yang W, Kishwar N, Rajput Z and Ariojo A 2006 Study of cellulolytic soil fungi and two nova species and new medium J. Zhejiang Univ. Sci. B. 7 459-466
[5] Zeilinger S, Gupta V K, Dahms T E S, Silva R N, Singh H B, Upadhyay R S, Gomes E V, Tsui C K-M, Nayaka S C 2016 Friends or foes? Emerging insights from fungal interactions with plants FEMS Microbiol. Rev. 40(2) 182-207
[6] Hoff J A, Klopfenstein N B, McDonald G I, Tonn J R, Kim M S, Zambino PJ, Hessburg P F, Rogers J D, Peever T L, Carris L M 2004 Fungal endophytes in woody roots of Douglas-fir (Pseudotsuga menziesii) and Ponderosa pine (Pinus ponderosa) For. Pathol. 34 255-271
[7] Talbot J M and Treseder K K 2010 Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53 169–179
[8] Upson R, Read D J and Newsham K K 2009 Nitrogen form influences the response of Deschampsia antarctica to dark septate root endophytes Mycorrhiza 20 1-11
[9] Newsham K K 2011 A meta-analysis of plant responses to dark septate root endophytes. New Phytol. 190 783–793
[10] Toju H, Satoshi Y, Sato H, Tanabe A S, Gilbert G S and Kadowaki K 2013 Community composition of root- associated fungi in a Quercus-dominated temperate forest: “codominance” of mycorrhizal and root-endophytic fungi Ecol. Evol. 3(5) 1281-1293
[11] Jumpponen A 2001 Dark septate endophytes are they mycorrhizal? Mycorrhiza 11 207-211
[12] Wilson B J, Addy H D, Tsuneda A, Hambelton S, Currah R S 2004 Phialocephala sphaeroides, sp. nov., a new species among the dark septate endophytes (DSE) from a boreal wetland in Canada Can. J. Bot. 82 607-617
[13] Knapp DG, Kovacs G M, Zajta E, Groenewald J Z, Crous P W 2015 Dark septate endophytic pleosporalean genera from semiarid areas Persoonia 35 87-100
[14] Caldwell B A, Jumpponen A, Trappe J M 2000 Utilization of major detrital substrates by dark septate root endophytes Mycologia 92 230-232
[15] Mandyam K, Loughlin T, Jumpponen A 2010 Isolation and morphological and metabolic characterization of common endophytes in annually burned tallgrass prairie Mycologia 102 813-821
[16] Surono and Narisawa K 2017 The dark septate endophytic fungus Phialocephala fortinii is a potential decomposer of soil organic compounds and a promoter of Asparagus officinalis growth Fungal Ecol. 28 1–10
[17] Surono and Narisawa K 2018 The inhibitory role of dark septate endophytic fungus Phialocephala fortinii against Fusarium disease on the Asparagus officinalis growth in organic source conditions Biol. Cont. 121 159-167
[18] Brundrett M C 2009 Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis Plant Soil 320 37-77
[19] Lambers H and Teste F P 2013 Interactions between arbuscular mycorrhizal and non- mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? Plant, Cell and Envir. 36 1911-1915
[20] Card S, Johnson L, Teasdale S, Caradus J. 2016 Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents FEMS Microbiol Ecol. 92 fiw114
[21] Joson L M, Coronel L M 1986 Isolation, screening and characterisation of cellulose-utilizing bacteria Phillip. J. Sci. 3 223-226
[22] Schwald W, Chan M, Breuil C, Saddler J N 1988 Comparison of HPLC and colorimetric methods for measuring cellulolytic activity Appl. Microbiol. Biotechnol. 28 398-403
[23] Chu D, Deng H, Zhang X, Zhang J, Bao J 2012 A simplified filter paper assay method of cellulase enzymes based on HPLC analysis Appl Biochem Biotechnol. 2012;167(1):190-196 doi:10.1007/s12010-012-9673-0
[24] Caldwell B A, Trappe J M, Jumpponen A 1996 Physiological characters of dark septate root endophytes ed T M Szaro, T D Bruns, T D (University of California, Berkeley: First International Conference on Mycorrhizae) p 19, 4-9 August 1996
[25] Jumpponen A, Trappe J M 1998 Dark septate endophytes: a review of facultative biotrophic root colonizing fungi New Phytol. 140 295-310
[26] Müller M M, Valjakkra R, Suokko A, Hantula J 2001 Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers Mol. Ecol. 10 1801-1810
[27] Snajdr J, Cjathaml T, Valaskova V, Merhautova V, Petrankova M, Spetz P, Leppanen K Baldrian P 2011 Transformation of Quercus petraea litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition FEMS Microbiol. Ecol. 75 291-303
[28] Lindahl B D, Tunlid A 2015 Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprophytes New Phytol. 205 1443-1447
[29] Behie S W and Bidochka M J 2014 Nutrient transfer in plant–fungal symbioses Trends in Plant Sci. 19(11) 734-740
[30] Jumpponen A, Mattson K G, Trappe J M 1998 Mycorrhizal functioning of Phia-locephala fortinii with Pinus contorta on glacier forefront soil: interactions with soil nitrogen and organic matter Mycorrhiza 7 261-265
[31] Narisawa K, Kawamata H, Currah R S, Hashiba T 2002 Suppression of Verticillium wilt in eggplant by some fungal root endophytes Eur. J. Plant Pathol. 108 103-109
[32] Narisawa K, Usuki F, Hashiba T 2004 Control of Verticillium yellows in Chinese cabbage by the dark septate endophytic fungus LtVB3 Phytopathol. 94 412-418
[33] Vohnik M, Albrechtova J, Vosatka M 2005 The inoculation with Oidiodendron maius and Phialocephala fortinii alters phosphorus and nitrogen uptake, foliar C: N ratio and root biomass distribution in Rhododendron cv Azurro Symbiosis 40 87-96
[34] Prade R A, Zohan D, Ayoubi P and Mort A J 1999 Pectins, pectinases and plant-microbe interactions. Biotechnol. Genet. Eng. Rev. 16 361-391
[35] Adeyoyo R O, Pletschke B I, Dames J F 2017 Improved endoglucanase production and mycelial biomass of some ericoid fungi AMB Expr. 7(15) 1-8
[36] Deswal D, Khasa Y P, Kuhad R C 2011 Optimization of cellulose production by a brown rot fungus Fomitopsis sp RCK 2010 under solid state fermentation. Bio Resour. Technol. 102(10) 6065 - 6072
[37] Liu D, Zhang R, Wu H, Xu D, Tang Z, Yu G, Xu Z, Shen Q 2011 Changes in biochemical and microbiological parameters during the period of rapid composting of dairy manure with rice chaff Bioresour Technol. 102 9040–9049
[38] Khan A L, Al-Harrasi A, Al-Rawahi A, AlFarsi Z, Al-Mamari A, Waqas M, et al. 2016 Endophytic fungi from Frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid PLoS ONE 11(6) e0158207
[39] Johnson N C, Graham J H, Smith F A 1997 Functioning of mycorrhizal associations along the mutualism–parasitism continuum New Phytol. 135 575–585
[40] Francis R and Read D J 1995 Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure Can J. Bot. 73(1) 1301–1309
[41] Johnson N C 1998 Responses of Salsola kali and Panicum virgatum to mycorrhizal fungi, phosphorus and soil organic matter: implications for reclamation J. Appl. Ecol. 35 86–94
[42] Olsson P A and Tyler G 2004 Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate J. Ecol. 92 808–815
[43] Usuki F, Narisawa K 2007 A mutualistic symbiosis between a dark septate endophytic fungus, 
Heteroconium chaetospira, and a nonmycorrhizal plant, Chinese cabbage Mycologia 99 175-
184
[44] Diene O, Wang W, Narisawa K 2013 Pseudosigmoidea ibarakiensis sp nov., a dark septate 
endophytic fungus from a cedar forest in Ibaraki, Japan Micr. Envir. 28 381-387
[45] Garcia-Romera I, Garcia-Garrido J M, Martinez-Molina E, and Ocampo J A 1990 Possible 
influence of hydrolytic enzymes on vesicular arbuscular mycorrhizal infection of alfalfa Soil 
Biol. Biochem. 22 149-152
[46] Garcia-Garrido J M, Tribak M, Rejon-Palomares A, Ocampo J A, and Garcia-Romera I 2000 
Hydrolytic enzymes and ability of arbuscular mycorrhizal fungi to colonize roots J. Exp. Bot. 
51 1443-1448
[47] Zuccaro A, Lahrmann U, Guldener U, Langen G, Pfiffi S, Biedenkopf D, et al. 2011 Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont Piriformospora indica PLoS Pathog. 7 e1002290
[48] Bartholdy B A, Berreck M, and Haselwandter K 2001 Hydroxamate siderophore synthesis by 
Phialocephala fortinii, a typical dark septate fungal root endophyte Biometals 14 33–42
[49] Menkis A, Vasiliauskas R, Taylor A F S, Stenlid J, Finlay R 2005 Fungal communities in 
mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, 
avessed by morphotyping, direct sequencing and mycelial isolation Mycorrhiza 16 33-41
[50] Lindahl B D, Ihrmark K, Boberg S E, Hogberg P, Stenlid J, and Finlay R D 2007 
Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest 
New Phytol. 173 611–620
[51] Taylor D L, Herriott I C, Long J, and O’Neill K 2007 TOPO TA is A-OK: a test of phylogenetic 
bias in fungal environmental clone library construction. Environ. Microbiol. 9 1329–1334
[52] Wilcox H E, Wang C J K 1987 Mycorrhizal and pathological association of dematiaceous fungi 
in roots of 7-month-old tree seedlings. Can. J. For. Res. 17 884-899
[53] Fernando A F, Currah R S 1996 A comparative study of the effects of the root endophytes 
Leptodonitium orchidicola and Phialocephala fortinii (fungi imperfecti) on the growth of 
some subalpine plants in culture Can. J. Bot. 74 1071-1078
[54] Grünig C R, Queloz V, Sieber T N, Holdenrieder O 2008 Dark septate endophytes (DSE) of the 
Phialocephala fortinii s1 - Acephala applanata species complex in tree roots: classification, 
population biology, and ecology. Can. J. Bot. 86 1355-1369
[55] Khastini R O, Ohta H, Narisawa K 2012 The role of a dark septate endophytic fungus, 
Veronaeopsis simplex Y34, in Fusarium disease suppression in Chinese cabbage. J. 
Microbiol. 50 618–624
[56] Khastini R O, Ogawara T, Sato Y, Narisawa K 2014 Control of Fusarium wilt in melon by the 
fungal endophyte, Cadophora sp. Eur. J. Plant Pathol. 139 339–348