Suppressive effects of *Pythium oligandrum* on soybean damping off caused by *P. aphanidermatum* and *P. myriotylum*

Xiaodong You, Joffroy Barraud* and Motoaki Tojo

**Abstract**

A strain of *Pythium oligandrum* isolated from soybean, grown in a commercial field in Osaka, Japan, was characterized by species identification and suppression of soybean damping off caused by *P. aphanidermatum* and *P. myriotylum*. The morphology and hyphal growth temperature of the *P. oligandrum* strain corresponded with those of the original description of *P. oligandrum*. rDNA-ITS sequences of the *P. oligandrum* strain were highly matched to those of the type strain of the species. The *P. oligandrum* strain was mycoparasitic toward *P. aphanidermatum* and *P. myriotylum*, and significantly suppressed soybean damping off caused by them. This is the first report of the effectiveness of *P. oligandrum* on soybean damping off pathogens.

**Key words:** biocontrol efficacy, *Pythium oligandrum*, soybean, damping off

**Introduction**

*Pythium oligandrum* is an effective biological control agent of damping off and root diseases caused by several soil-borne pathogens, including phytopathogenic *Pythium* species (Martin and Hancock, 1987; Hase et al., 2006). It interacts directly with the fungal pathogens through mycoparasitism, antibiosis, nutrient and space competition, and/or indirectly by inducing resistance in the plants (Benhamou et al., 1999; Takenaka et al., 2003). Antagonistic effects of *P. oligandrum* against phytopathogenic *Pythium* species may differ significantly among species and isolates of the target pathogens (Foley and Deacon, 1986). For example, *P. aphanidermatum*, *P. graminicola* and *P. vexans* are not always received with mycoparasitism of *P. oligandrum* (Berry et al., 1993; Benhamou et al., 1999; Laing and Deacon, 1991). Le (2016) demonstrated that *P. oligandrum* isolated from a ginger field was more virulent to a ginger-origin isolate than a capsicum origin isolate of *P. myriotylum*. These reports suggest that sharing of the same origin is important to obtain a high effectiveness of *P. oligandrum* on its application in crop protection. *P. oligandrum* has been reported to be nonpathogenic toward soybeans and is frequently associated with soybean roots in the field (van der Plaats-Niterink, 1981; Kirkpatrick et al., 2006). However, biocontrol efficacy of the soybean-origin *P. oligandrum* against soybean pathogenic *Pythium* has never been reported.

*Pythium aphanidermatum* and *P. myriotylum* have been recognized as aggressive pathogens that cause soybean damping off (You and Tojo, 2017; Tomioka et al., 2013). Although chemical treatments, such as fungicide-seed coating and/or soil fumigations, are effective in managing the disease, frequent use of chemical treatments cause the emergence of fungicide-resistant strains, and risk human health as well as the surrounding environment (Bradley, 2008; Becker et al., 1998). An alternative strategy to control soil-borne plant diseases is the application of biocontrol agents, such as rhizobacteria and rhizofungi (Léon et al., 2009; John et al., 2010).

Despite indicators of the potential of *P. oligandrum* for control of soybean damping off, no comprehensive evaluation of the species has been conducted on its effectiveness on the disease. *P. oligandrum*, strain D11, was isolated from the rotted root of a soybean seedling from a converted paddy field in Osaka Prefecture, Japan, in June, 2016 by the authors. The strain showed suppressive effects on soybean...
damping off caused by *P. aphanidermatum* and *P. myriotylum* in pot experiments. Here we describe the morphological and molecular characteristics of *P. oligandrum* strain D11. This paper is the first to describe the suppressive effects of *P. oligandrum* on soybean damping off pathogens.

**Materials and Methods**

**Morphological and molecular characteristics of Pythium oligandrum strain D11**

Morphological identification of strain D11 was based on the keys of van der Plaats-Niterink (1981). Hyphal growth rates of strain D11 at different temperatures were determined on potato carrot agar (PCA) according to the method described by Tojo et al. (2012). The rDNA-ITS regions of the strain was amplified and sequenced with primers ITS4 and ITS5 (White et al., 1990).

**Soybean damping-off pathogens used**

The pathogens used were *Pythium aphanidermatum* strain D1 from Sakai city, Osaka and *P. myriotylum* strain OPU894 from Toyama city, Toyama. Both strains were recovered from damped-off soybean seedlings grown in converted paddy fields. *P. aphanidermatum* strain D1 was identified previously (You and Tojo, 2017). Species identifications of *P. myriotylum* strain OPU894 was performed by morphology (data not shown) and sequencing of the ribosomal ITS region. The GenBank Accession Nos. of the ITS regions are MF769579 for *P. aphanidermatum* strain D1 and MH707061 for *P. myriotylum* strain OPU894.

**Effects of Pythium oligandrum strain D11 against damping off of soybean**

Biocontrol efficacy of *P. oligandrum* strain D11 was performed against soybean damping off caused by *P. aphanidermatum* strain D1 and *P. myriotylum* strain OPU894 by the method of Kobayashi et al. (2010) with several modifications (Fig. 1). Briefly, the three *Pythium* strains were cultured on autoclaved bentgrass seeds (Tojo et al., 1993) at 28°C for one week. The *Pythium* infested bentgrass seeds were diluted by a commercial nursery soil (Takii-ikubyoubaido; Takii Seed Co., Ltd., Kyoto, Japan) in concentrations of 3%, 0.025% and 0.05% (w/v) for *P. oligandrum* strain D11, *P. aphanidermatum* strain D1 and *P. myriotylum* strain OPU894, respectively. A single layer of a water-soluble paper (Scottie toilet tissue, Nippon Paper Crecia, Tokyo, Japan) was cut into a disk shape (7 cm diameter) and was placed on 90 ml of the *P. aphanidermatum* or *P. myriotylum* infested soil in a plastic pot (inner diameter 7 cm, inner depth 6.5 cm). Sixty ml of the *P. oligandrum* infested soil was layered on the paper disk and seven soybean seeds (cv. EzoMidori) were placed into it. Then, 30 ml of the *P. aphanidermatum* or *P. myriotylum* infested soil was placed on the *P. oligandrum* infested soil. Each pot was irrigated daily with tap water. Both pathogens were mainly caused pre-emergence damping off in the present study. Therefore, the control effects were evaluated by the number of emerged seedlings at 10 days after sowing. The experiment was arranged in a randomized block, with 7 replications for each combination of the *P. oligandrum* strain and the two pathogenic *Pythium* strains. All statistical analyses were performed with JMP Version 8 (SAS Institute, Cary, NC, USA). The data were tested with analysis of variance followed by the Turkey HSD test (*P* < 0.05).

**Mycoparasitism of Pythium oligandrum strain D11 against P. aphanidermatum and P. myriotylum**

The mycoparasitism activity of *P. oligandrum* strain D11 against *P. aphanidermatum* strain D1 and *P. myriotylum* strain OPU894 was assayed in a dual-culture test as follows. Each mycelial square (4 mm diameter) collected from the edge of a growing colony of one of the three *Pythium* species on corn meal agar (CMA, Becton Dickinson and Company, Franklin Lakes, USA) was transferred to a cellophane film disk (90 mm diameter) placed on 1/4 CMA.

![Fig. 1. Schematic representation of the experimental design.](image-url)
medium in a Petri dish (90 mm diameter). *P. oligandrum* and the two pathogenic *Pythium* species were placed 6 cm apart on the opposite sides of the surface of the cellophane film disk. After incubation for 3 days at 25°C, a strip of the film (15 mm²) was removed from the crossing zone between the mycelia and was observed for mycoparasitism under a compound light microscope.

**Results**

**Morphological and molecular characteristics of *Pythium oligandrum* strain D11**

*Pythium* strain D11 was identified as *P. oligandrum* based on its morphological and molecular characteristics. The observed morphological characteristics were as follows: main hyphae was up to 6 μm wide. Sporangia were mostly intercalary, occasionally terminal, consisting of one or more subglobose elements with connecting filamentous parts (Fig. 2). Oogonia were terminal or intercalary 20–26 (av. 24) μm in diameter (Fig. 2). Antheridia were mostly lacking, but sometimes 1 or 2 per oogonium. Oospores were aplerotic and 15–22 (av. 18) μm in diameter (Fig. 2). The thickness of oospore walls ranged from 0.6–1.6 (av. 1.2) μm. The optimum temperature for mycelial growth of strain D11 on PCA was 28–34°C. The maximum and minimum temperatures for mycelial growth were 37°C and 7°C, respectively. The daily growth rate at 25°C was 26.9 mm per day. Molecular study based on sequence data from the rDNA-ITS showed that strain D11 had 100% similarity to several strains of *P. oligandrum* in Genbank, for example, strain Oth2 (KJ908710), isolated from the rhizosphere of vines (Gerbore et al., 2014). The sequence is available in the DDBJ/EMBL/GenBank database under accession number MH571938. *P. oligandrum* strain D11 is deposited at the National Institute of Agrobiological Sciences, Japan as accession number MAFF 246894.

**Effects of *Pythium oligandrum* strain D11 against pre-emergence damping off of soybean**

*P. oligandrum* strain D11 suppressed the damping off caused by *P. aphanidermatum* and *P. myriotylum*. The *P. oligandrum* strain significantly (*P* < 0.05) increased the seedling stands to 67% from 10% and 63% from 32% in the *P. aphanidermatum* and *P. myriotylum* infested soils, respectively (Figs. 3 and 4).

**Mycoparasitism of *Pythium oligandrum* strain D11 against *P. aphanidermatum* strain D1 and *P. myriotylum* strain OPU894**

The dual-culture tests demonstrated that *P. oligandrum* strain D11 is mycoparasitic against both *P. aphanidermatum* strain D1 and *P. myriotylum* strain OPU894. The hyphal contact of *P. oligandrum* against pathogens was started at 44 and 52.5 hour after their inoculations of *P. aphanidermatum* and *P. myriotylum*, respectively. Hyphae of the *P. oligandrum* strain coiled and penetrated hyphae of both the *P. aphanidermatum* and *P. myriotylum* strains in their contact zones within one day after the contacts (Fig. 5). In the penetrated hyphae, cytoplasm had partially disappeared.

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**Fig. 2.** Morphology of *Pythium oligandrum* strain D11 formed in a grass blade culture.

Discussion

The present study revealed that *P. oligandrum* strain D11, isolated from a soybean root, can suppress soybean damping off caused by *P. aphanidermatum* and *P. myriotylum* under greenhouse conditions. The mycoparasitism of the *P. oligandrum* strain against the *P. aphanidermatum* and *P. myriotylum* strains was also clarified here. These results agree with previous studies showing that *P. oligandrum* can be an antagonist against these two pathogens (Benhamou et al., 1999; Le, 2016). Berry et al. (1993) reported, on the other hand, that *P. oligandrum* was rarely mycoparasitic to *P. aphanidermatum* but was sometimes parasitized by

Fig. 3. Effects of *Pythium oligandrum* strain D11 on seedling stands of soybean grown in a commercial nursery soil infested with *P. aphanidermatum* (A) and *P. myriotylum* (B) at 10 days after sowing. Bars indicate standard error (*N* = 7). Treatments with different letters indicate significant difference at the 0.05 level, according to Tukey’s HSD test (*P* < 0.05).

Fig. 4. Effects of *Pythium oligandrum* strain D11 on soybean seedlings grown in a commercial nursery soil infested with *P. aphanidermatum* and *P. myriotylum* at 10 days after sowing. A: a. Noninoculated, b. *P. oligandrum*, c. *P. aphanidermatum*, d. *P. oligandrum* + *P. aphanidermatum*. B: a. Noninoculated, b. *P. oligandrum*, c. *P. myriotylum*, d. *P. oligandrum* + *P. myriotylum*.

Fig. 5. Mycoparasitic interactions between A: *Pythium oligandrum* (PO) and *P. aphanidermatum* (PA). B: *P. oligandrum* (PO) and *P. myriotylum* (PM) in dual culture. Bar = 50 μm.
the pathogen, which is different from our results. This may be due to that both the isolates of \textit{P. oligandrum} and \textit{P. aphanidermatum} used in this study were originally obtained from soybean roots. Berry et al. (1993) used the \textit{Pythium} isolates from different host origins. Foley and Deacon (1986) reported that the isolates within the same \textit{Pythium} species might exhibit various degrees of susceptibility to the mycoparasite \textit{P. oligandrum}. The soil-borne pathogens are most vulnerable to mycoparasitism by \textit{P. oligandrum} when the \textit{P. oligandrum} was originally recovered from the same rhizospheres as of the pathogens (Gerbore et al., 2014). The results from present and the previous studies suggest that sharing of host plants may be important for the disease suppressiveness of \textit{P. oligandrum}. Further study is needed to determine whether the other strains of \textit{P. oligandrum} isolated from the different host plants or rhizospheres show the disease suppressiveness.

\textit{P. oligandrum} was thought to potentially be an effective biocontrol agent for leguminous crops because no pathogenicity of the species has been reported for any leguminous crops including soybean, pea, red kidney bean, and alfalfa. Also, suppressive effects of \textit{P. oligandrum} have been reported on pea damping off caused by \textit{Pythium} (Brozova, 2002), however, no evaluation has been performed on biocontrol efficacy of \textit{P. oligandrum} on soybean diseases. This is the first report of the effectiveness of \textit{P. oligandrum} on soybean damping off pathogens. Considering that the combined application of the biocontrol agents with suitable amendments, such as compost or vermicompost, can significantly enhance the process of colonization and survival of the inoculants, thus enhancing the biocontrol activity (Sahni et al., 2008; Singhai et al., 2011). Our further work will determine whether the \textit{P. oligandrum} alone or \textit{P. oligandrum} combined with vermicompost is effective in suppressing soybean damping off in fields.

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**References**