Isolation and Evaluation of Fungal Endophytes for the Suppression of Fusarium Diseases

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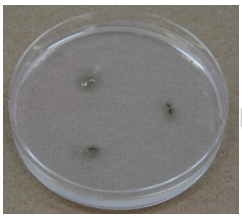
Abstract

In order to obtain biocontrol agents against Fusarium diseases, fungal endophytes were isolated from plants (roots, leaves-stems, and winter buds) and soil samples collected from natural forest sites in Ibaraki prefecture in Japan. Totally, eight fungal endophytes have been isolated during this study and tested tested for their ability to suppress Fusarium wilts in melon, tomato and asparagus, respectively known as *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *lycopersici* and *F. proliferartum*. Results showed two isolates *Meliniomyces variabilis* (isolate I.1-2-1) and unidentified DSE (isolate 312-6) have potential in the control of Fusrium diseases in lab conditions. *M. variabilis* could tolerate the occurrence of *F. oxysporum* in melon and tomato; and *F. proliferartum* in asparagus.

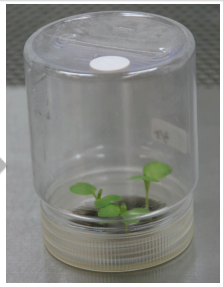
Background

Fungal endophytes cause symptomless infection to host plants. Some fungal endophytes are associated with plant protection abilities and as such can be used as biocontrol agents. For this reason, we have initiated this study to collect new endophyte isolates to use as biocontrol agents against Fusarium wilt in melon, tomato and asparagus. The ability of the pathogens to develop several races known as formae specialae (f. sp), make it difficult to find efficient control method. The development of efficient control methods against Fusarium diseases is an urgent necessity, specially in asparagus where no control method is available. Fungal endophytes have already proven effectiveness as biocontrol agents for suppressing plants diseases. However, no study is reported on fungal endophyte able to control these diseases. Consequently, we investigated this study to provide efficient endophyte biocontrol agents for the suppression the specific pathogens in melon, tomato and asparagus.

Endophytes isolation Methodology



Plant rot fragments plated on cornmeal for fungal isolation.



Pathogenicity testing on Chinese cabbage.

Endophytes Isolation results

In this study, totally, eight fungal endophytes were selected (Table 1).

Table 1. Eight fungal endophytes tested during this study

Isolate Nº	Species	Source	Origin
41-1	*Helminthosporium velutinum*	Prinus tree	Buds
281-10	*Leptodontidium orchidicola*	Goldenrod weed	Roots
312-6	unidentified	unidentified	Roots
298-3	*Pseudocercospora abelmoschi*	*Stewartia pseudocamellia*	Buds
309-4	unidentified	unidentified	Roots
309-8	unidentified	unidentified	Roots
I.4-2-1	*Pseudosigmoidea* sp.	Tomato	Soil
I.1-2-1	*Meliniomyces variabilis*	Tomato	Soil

The major findings are *H. velutinum* and *Pseudosigmoidea* sp. reported in here for the first time as endophytes (Fig. 2 A &B, respectively).

Leptodontidium orchidicola and Meliniomyces variabilis ((Fig. 2 C & D, respectively).) are already reported as endophytes but this is the first time they reported from Japan, indicating that the distriction of dark sepate endophytes can be larger than previously thought.

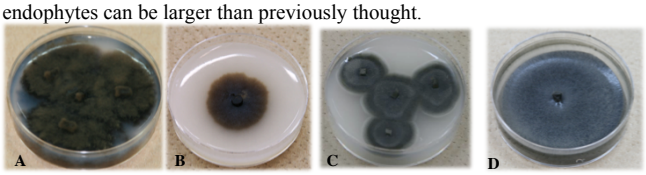


Fig. 2. Fungal colonies of *Helminthosporium velutinum* (A), *Pseudosigmoidea* sp. (B), *Meliniomyces variabilis* (C), and *Leptodontidium orchidicola* (D) on oatmeal agar.

Concluding remarks

These results represent important achievements and show clearly that our new fungal endophyte isolates have a potential to suppress Fusarium diseases in the lab conditions. These results are most important as for asparagus, there is no control method yet available. Through endophytes, there is possibility of breaking the resistance problem resulting from the multiple races the different Fusarium account. Moreover, we confirm that DSE isolated out of their previously reported area can still have useful functions of stress tolerance.

Ability of endophytes to use different nitrogen sources

Species of *Pseudosigmoidea* could use amino aciods (Phe., and Leu.) to support their host plants growth (Fig. 3).

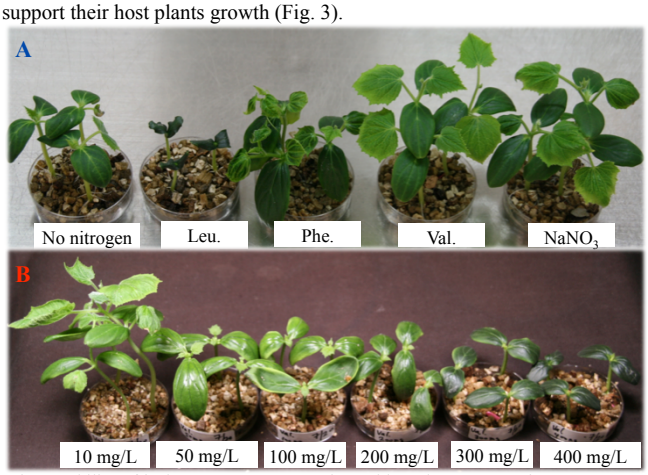


Fig. 3. Ability of isolate I.4-2-1 to use amino acids and $NaNO_3$ as nitrogen sources. **A,** Effects of the 3 amino acids on the growth of cucumber. **B,** Effects of the val. concentration on the cucumber seedlings growth.

Endophytes tests for control of Fusarium diseases

Two isolates *Meliniomyces variabilis* (isolate I.1-2-1) and an unidentified DSE (isolate 312-6) showed potential for suppressing Fusarium wilt in melon Fig . 4A, tomato (not shown) and asparagus (Fig. 4B, in lab conditions. as shown in Fig . 2 with the asparagus.

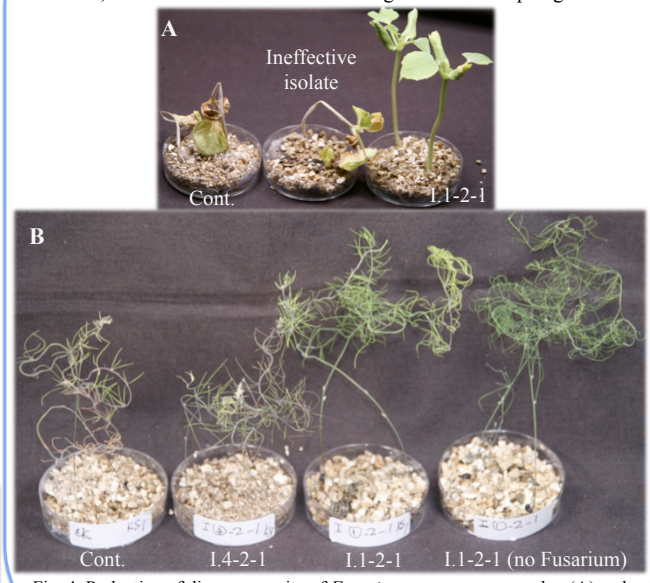


Fig. 4. Reduction of disease severity of *Fusarium oxysporum* on melon (A) and *F. proliferatum* (KS-1) in asparagus (B) by *Meliniomyces variabilis* (isolate I.1-2-1).