# In vitro evaluation for biocontrol potential of dark-septate endophytes in Asparagus disease

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## Abstract

We conducted the study for the purpose of controlling Fusarium disease in asparagus with the use of dark-septate endophytes (DSE). Firstly, we isolated pathogenic fungi from young buds of asparagus to investigate factors of Fusarium disease in Field Science Center (FSC), Ibaraki university. Crown rot caused by *F. proliferatum* is the main factor of Fusarium disease in FSC. Secondly, we screened DSE isolates that are effective in suppressing crown rot in Asparagus. DSE isolates suppress effectively the disease, especially J2PC2 identified as *Phialocephala fortinii* also suppressed root rot caused by *F. oxysporum*.

## Introduction

In asparagus cultivation, Fusarium crown and root rot caused by *Fusarium oxysporum* and *F. proliferatum* are serious problem, and difficult to control. Fungal endophytes are microorganisms inhabiting in plant tissues, without causing injury to host plant. Some endophytes associate host plant growth, stress tolerance and to disease and pest resistance . We proposed dark-septate root endophytes (DSE) which occur in the roots of diverse plant species may be able to suppress the disease of their host plants. Therefore, we performed a screening experiment for endophytic fungal candidates that effectively suppress Fusarium disease in Asparagus.

## Investigation of pathogenic fungal species

Totally 78 pathogenic isolates were obtained from FSC, and classified into 12 groups based on morphological characteristics. Twelve isolates from each group were tested for pathogenicity in asparagus.
Isolates identified as *F. proliferatum* had a high isolation rate, and many of them indicated pathogenicity for seedlings of asparagus.

Fig. 1. Typical yellow symptoms of Fusarium disease in FSC .

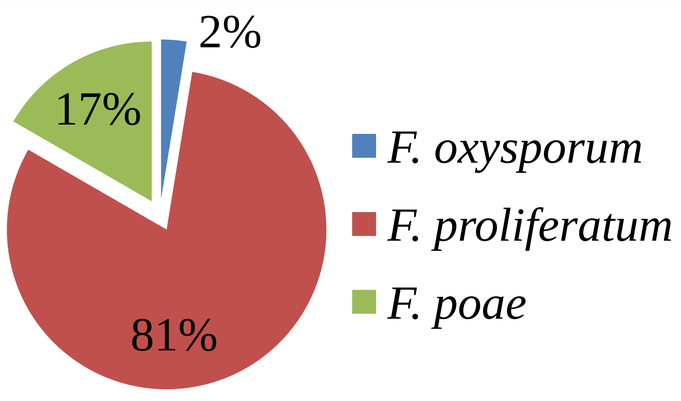


Fig. 2. Fungal isolation rate form young buds of asparagus in FSC.

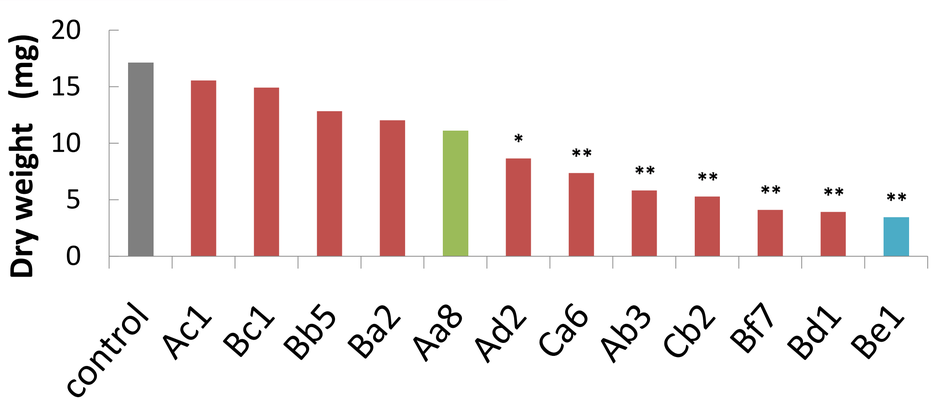


Fig. 3. Dry weight of aerial parts of asparagus seedlings 6 weeks after fungal isolates inoculation. *p<0.05, **p<0.01: significantly different from control (Dunnet test).

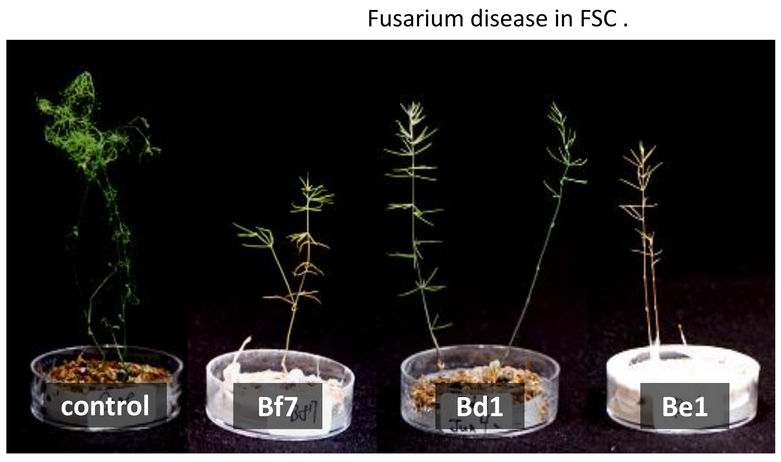


Fig. 4. Results of isolates pathogenicity tested in asparagus.

## Screening of DSE for suppressing disease

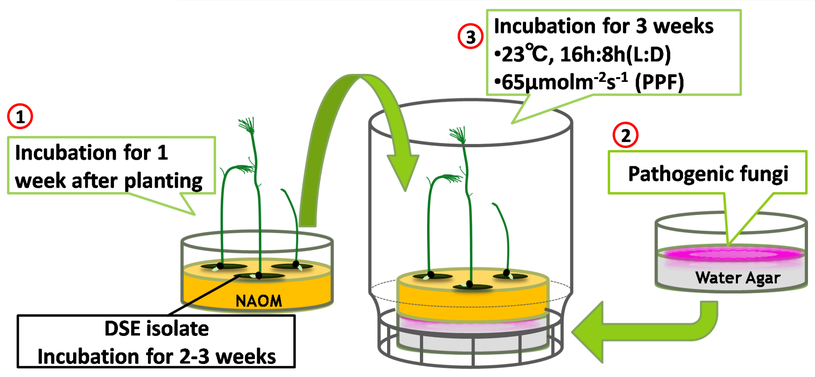


Fig.5. Method of disease suppression trial with agar medium.

NAOM medium[$MgSO_4 \cdot 7H_2O$ (Wako), 1g; $KH_2PO_4$ (Wako), 1.5g; Oatmeal, 10g; Agar (Wako), 18g; Nature-Aids(Sakata Seed Co.)(filter sterilized), 6.6ml $L^{-1}$]

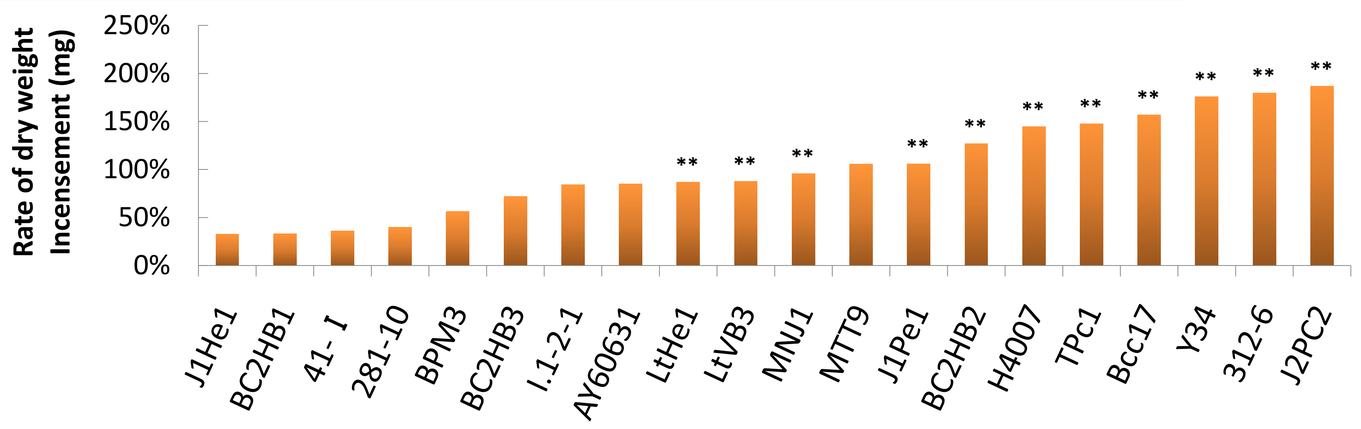


Fig. 6. Results of screening of DSE isolates that suppressing *F. oxysporum* effectively in asparagus seedlings.
**p<0.01: significantly different from control (Dunnet test).

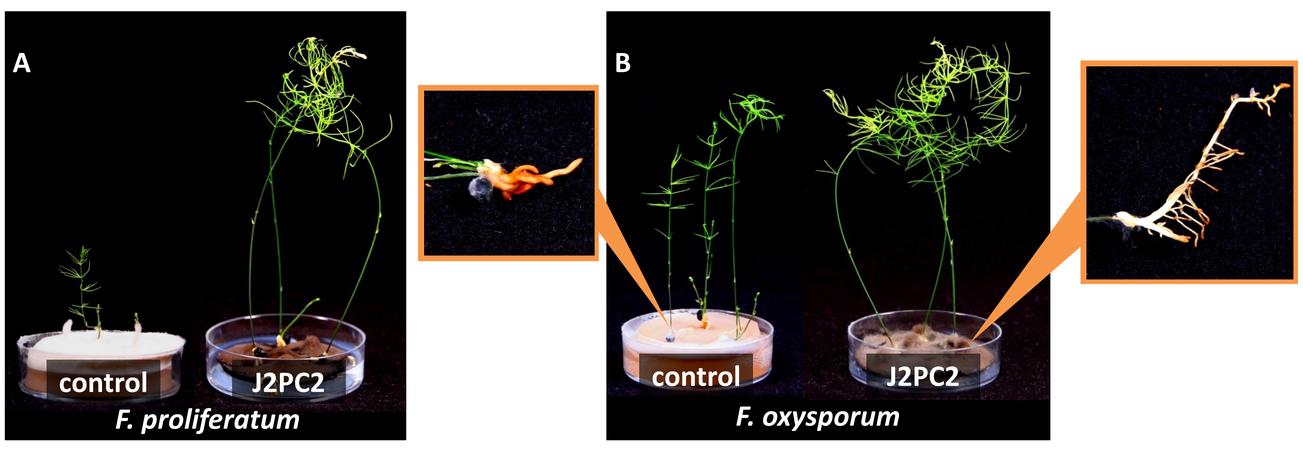


Fig. 7. Results of disease suppression trial with DSE isolate J2PC2 and Pathogenic fungi *F. proliferatum* (A), *F. oxysporum* (B).

J2PC2 isolate identified as *Phialocephala fortinii* was indicated high suppression effect for *F. proliferatum* as well as *F. oxysporum*.

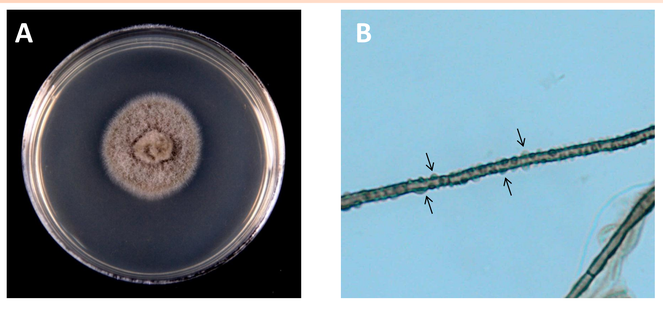


Fig. 8. One week Culture of J2PC2 on CMMY medium (A). Typical characteristic morphology of the hyphal structure (B).

## Conclusion and Future study

DSE inoculated plants showed higher dry weight than those of control plants. The effect was differed among endophytic fungal candidates. J2PC2 isolate indicated the highest effect among the isolates.
We will carry out more pre-screening on DSE, and provide the suppression values of selected isolates against different pathogenic isolates.
We will also determine the nutritional conditions for the effectiveness of colonization of DSE in the roots of host plant and their suppression of Fusarium disease. The future goal is to produce asparagus seedling inoculated by DSE which is resistant to Fusarium disease commercially.