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Screening of the endophytic bacteria with antifungal effect against Anthracnose in *Panax ginseng*

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ABSTRACT

Background : *Panax ginseng* a crop cultivated for 4 to 6 years in the same field and is exposed to various biological and abiotic stresses. In particular, it is vulnerable to infection by fungal pathogens in the aboveground parts, which can lead to severe damage and even root decay, resulting in a decrease in yield. Among fungal pathogens, Anthracnose caused by *Colletotrichum gloeosporioides* is a common disease affecting the aboveground parts of ginseng. In this study, we screened the endophytic bacteria strains with antagonistic effect against *C. gloeosporioides*.

Methods and Results : The endophytic bacteria of ginseng were isolated from various tissues, ages, and species. To screen antagonistic strains, we performed a dual culture assay against *C. gloeosporioides* in *in vitro*. Among 311 strains, 77 strains inhibited the hyphal growth of *C. gloeosporioides*. Especially four strains belonging to *Bacillus* genus effectively inhibited the hyphal growth of the pathogen. To evaluate the efficacy of biocontrol against *C. gloeosporioides*, we performed pot experiments. The 2-year-old ginseng seedlings were cultivated for 4 weeks in a greenhounse and each bacterial suspension were applied on the leaves with different concentration (OD_{600} = 1.0, 0.1. 0.01). After 24h, *C. gloeosporioides* were inoculated and one week after inoculation, the disease index was calculated. Among four strains, PgBE151 effectively inhibited disease symptom compared with DW control.

Conclusion : Based on our results, it will be help to understand the application of *B. amyloliquefaciens* as a biocontrol agent for ginseng protection from the pathogen, *C. gloeosporioides*.

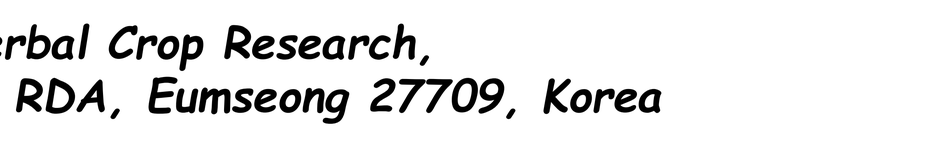
RESULTS

Molecular identification of selectec isolates.

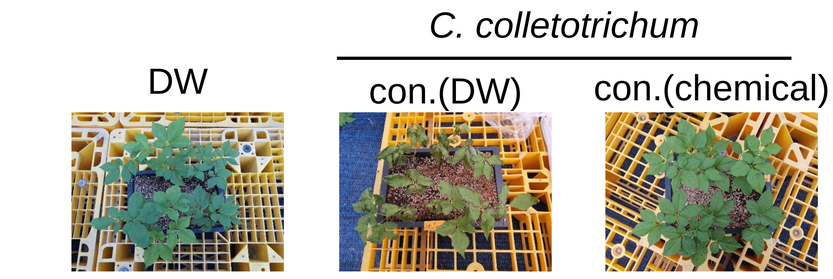
Strain	Isolates	16S rRNA(bp)	Identity(%)
1	PgBE14	*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42	99
2	PgBE25	*Bacillus subtilis* strain DSM 10	99
3	PgBE71	*Pseudomonas azotoformans* strain NBRC 12693	99
4	PgBE83	*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42	99
5	PgBE85	*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42	99
6	PgBE89	*Pseudomonas puttida*	99
7	PgBE94	*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42	99
8	PgBE95	*Bacillus thuringiensis strain IAM 12077*	99
9	PgBE96	*Pseudomonas koreensis strain Ps 9-14*	99
10	PgBE99	*Bacillus amyloliquefaciens subsp. plantarum strain FZB42*	99
11	PgBE151	Bacillus amyloliquefaciens subsp. plantarum strain FZB42	99
12	PgBE182	*Pseudomonas fluorescens Pf0-1 strain Pf0-1*	100
13	PgBE188	*Rhizobium soli strain DS-42*	99
14	PgBE215	*Pseudomonas azotoformans strain NBRC 12693*	99
15	PgBE220	*Pseudomonas koreensis strain Ps 9-14*	99
16	PgBE247	*Bacillus cereus* ATCC 14579	99
17	PgBE253	*Pseudomonas frederiksbergensis* strain DSM 13022	99
18	PgBE262	*Micrococcus luteus* strain NCTC 2665	99

SUMMARY

- Among 311 ginseng endophytes, 18 strains inhibited the hyphal growth of airborne pathogens such as *Botrytis cinerea*, *Altarnaria panax* and *Colletotrichum gloeosporioides*.
- Four strains belonging to the *Bacillus* genus showed excellent antifungal activity compared to other isolates.
- The biocontrol activities varied based on the concentration of the treated strains.



Antifungal activity of the isolates against air-borne pathogen, *Colletotrichum gloeosporioides* in *in vitro*. Pathogens were cultured on a PDA plate for 7 days at 20°C in the dark. After 2 days of pre-cultivation of the indicated fungal pathogens, 10 μl of each suspension with a concentration of OD_{600}=0.1 (2), 1.0 (3) and distilled water (1) were dropped on a PDA plate. Each bacterial suspension was applied 1.5−2.0 cm away from the fungal disc. DW was used as a control. Images were obtained after 2−3 days co-cultivation.



Disease symptoms on ginseng leaves were observed one week post inoculation with *C. gloeosporioides* (1.0 x 10^4 spores/ml). DW and a pesticide were used as controls.