J. Microbiol. Biotechnol. (2002), **12**(6), 1022–1025

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Antifungal Activities Against *Plasmodiophora brassicae* Causing Club Root

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Received: November 5, 2002
Accepted: November 26, 2002

Abstract Club root is one of the major diseases that occur in crucifers. It is caused by *Plasmodiophora brassicae*. In order to discover microbial biopesticides against *P. brassicae*, forty-eight *Streptomyces* isolated from soil were screened. Among these, three strains showed excellent pesticidal activities. We report results on *in vivo* screening with fermentation broths of these strains and identification of the strain taxa.

Key words: *In vivo* antifungal activities, *Plasmodiophora brassicae*, *Streptomyces*, club root

One of the diseases occurring on crucifers, club root, is caused by *Plasmodiophora brassicae*, which inhabits soil. Historically, club root was first reported in the 13th century in Europe, and has been found in over 60 infected genera of crucifers including cabbage, broccoli, and turnip. In Korea, it is one of two major diseases that occur in cabbage. Before 1995, black-leg caused by *Erwinia carotovora* subsp. was the major disease, however, today club root has become the major plague. *P. brassicae* passes winter as resting spores which germinate in a favorable condition such as cool, wet, and acidic soils. The germinated spore produces a zoospore resulting in an infection of host root hairs where plasmodia are produced. As a result, a zoosporangium is developed from the plasmodium. The zoosporangia release the secondary zoospores which pass into roots, tissues, and underground stems of hosts. Finally, such infections cause the club root to decay host tissues.

Since resting spores can survive at least seven or eight years in soil, the crop rotation is the best way to eliminate them. However, they can be disseminated by the transport of the infected soil during cultivation of other crops, so that the complete elimination of resting spores from soils is very difficult. Although there are a few chemicals known to protect against *P. brassicae*, biopesticides have attracted our interest because of the rejection of synthetic chemical pesticides by consumers. The advantage of a biopesticide such as *Bacillus thuringiensis* is that it can be lethal and specific. In particular, if the microorganisms used as a biopesticide can survive in soils for a long period of time, they are very suited for perventing infection by resting spores of *P. brassicae*.

Streptomyces has been studied because of its diversity of biotransformation and secondary metabolites [8, 10, 12]. Thus, about two-thirds of all the clinically important antibiotics now being used actually belong to secondary metabolites which are produced by *Streptomyces*. In order to discover a microbial biopesticide against *P. brassicae*, forty-eight *Streptomyces* isolated from soil samples on Jeju island were screened. Since the fermentation broth of the strain was used for the screening process, it was considered that the secondary metabolites produced by the strain showed some activity.

Soil samples were collected during 2001 at several sites near Hanla Mountain in Jeju island, Korea. Samples were used as substrates for isolating actinomycetes that exhibited antifungal activities. Detailed procedures for isolation followed the method previously published by Kim *et al.* [2]. Colonies showing the typical characteristics of actinomycetes were selected and transferred to Bennet agar medium that was adjusted to pH 7.2, and cultured for 10 days at 28°C. Samples of each colony from the agar were inoculated into 30 ml of Bennet broth in a 100-ml capped tube and cultured in a shaking incubator at 28°C for 30 days. Fermentation broths were used for screening tests.

Samples were tested for *in vivo* antifungal activities against *Plasmodiophora brassicae* Woron. The crop used for testing was *Brassica campestris* subsp. napus var.

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pekinensis, cv Hukjinju. It was obtained from a horticultural station. When the crop grew into the second leaf stage, *P. brassicae* was added. In order to attack with a club root, resting spores were mixed with soil to give final concentration of 1×10^6 spores/ml. Dried soil was placed on the plastic pot and the second leaf stage crop was placed in. The soil containing resting spores was added on the crop. Ten ml of the fermentation broth was poured on the pot, and test crops were grown in a greenhouse at 25(±5)°C for 21 days. The fungal control was analyzed based on the following index: 0=no clubs, 1=small galls formed on lateral roots, 2=galls formed on lateral roots or small galls formed on the main roots, and 3=many big galls formed on lateral and main roots. Since the control does not include any fermentation broth, it should show the index 3 [4].

Among forty-eight actinomycetes screened, three strains showed 100% control value, as listed in Table 1. Fluazinam was used as a reference. It showed 67% control value at 125 ppm and 100% control value at 500 ppm. Figure 1 shows the root of the crop used for testing, *Brassica campestris* subsp. napus var. pekinensis, cv Hukjinju, not treated (left) or treated with *P. bracissicae* (right). Strains BG2-17, BG2-19, and BG2-43 showed 100% control value. As mentioned earlier, 100% of the control value denotes that there were no clubs. Figure 2 shows the root of the crop treated with BG2-17 after being infected by *P. bracissicae*. The results obtained by treatment with other strains, except for three strains mentioned above, showed indices between 0.5 and 2.5. In order to compare the severity of infection *vs.* different indices, the results obtained by treatments with BG2-43, BG2-58, and B-21, whose indices were 0, 1, and 2, respectively, are shown in Fig. 3.

Table 1. The activities of fermentation broths of strains tested against *Plasmodiophora brassicae.*

Strain	Index*	Control value (%)	Strain	Index*	Control value (%)
BG2-4	1.5	50	H183-1	1.5	50
BG2-15	2.0	33	2H-1	1.5	50
BG2-16	0.5	83	2H-2	0.5	83
BG2-17	0.0	100	2H-3	1.5	50
BG2-19	0.0	100	2H-4	0.5	83
BG2-21	2.0	33	2H-6	0.5	83
BG2-22	0.5	83	2R-6	1.5	50
BG2-41	0.5	83	S-27	1.5	50
BG2-43	0.0	100	S-29	1.5	50
BG2-48	0.5	83	S-43	1.5	50
BG2-54	0.5	83	S-79	1.0	67
BG2-55	0.5	83	S-115	0.5	83
BG2-58	1.0	67	2S-1	1.0	67
BG2-65	1.0	67	2S-11	0.5	83
B-1	1.0	67	2S-15	0.5	83
B-13	2.0	33	2S-17	0.5	83
B-21	2.0	33	2S-25	0.5	83
B-30	1.5	50	2S-26	1.0	67
B-55	1.0	67	2S-27	0.5	83
B-59	0.5	83	2S-28	1.5	50
B-78	0.5	83	2S-29	0.5	83
B-81	0.5	83	2S-30	1.0	67
H1-4	0.5	83	2S-31	2.0	33
H53-2	1.0	67	Y-56	2.5	17

*Index: 0=no clubs, 1=small galls formed on lateral roots, 2=galls formed on lateral roots or small galls formed on the main roots, 3=many big galls formed on lateral and main roots.
The control gave the index of 3.0 and 0% control value.
Fluazinam at 125 ppm and 500 ppm used as a reference gave the index of 1.0 and 67% control value, and 0.0 and 100%, respectively.

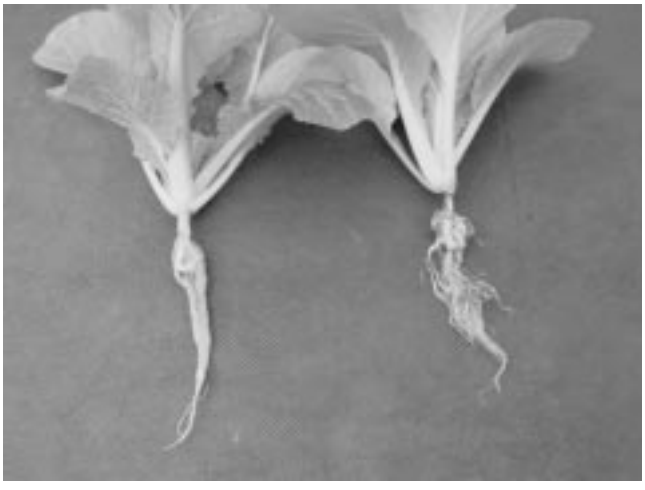

Fig. 1. The roots of the crop used for testing, *Brassica campestris* subsp. napus var. pekinensis, cv Hukjinju, not treated (left) or treated (right) with *P. bracissicae.*

The strains BG2-17, BG2-19, and BG2-43 showing 100% of the control value were identified as *Streptomyces* sp. on the basis of partial 16S rDNA [5, 6, 13]. The

Fig. 2. The root of the crop treated with BG2-17 after being infected by *P. bracissicae*.

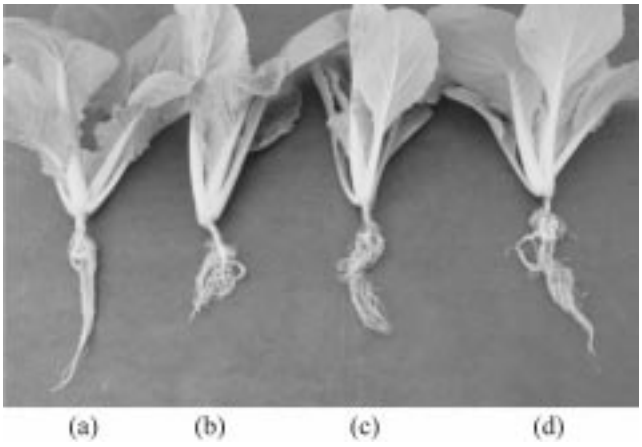

Fig. 3. The results obtained from treatments with (a) BG2-43 (index=0), (b) BG2-58 (index=1), (c) B-21 (index=2), and (d) control (index=3).

detailed procedures for 16S rDNA analysis followed the previous method published by Kim *et al.* [2, 3]. 16S rDNA sequences (452-bp) of the strain BG2-17, identified from GenBank by the BLAST program, showed the highest homology (99% identity) with *Streptomyces lavendulae* [7, 9]. A comparison of the strain BG2-17 with *Streptomyces lavendulae* is shown in Fig. 4. The evolutionary tree constructed using the PHYDIT program indicated that the strain BG2-17 was related to *S. bikiniensis*, *S. lipmanii*, and *S. subrutilus* with a high bootstrap value [1, 11].

The 462 bp of strain BG2-19 showed the highest homology (98%) with *S. panayensis*. A comparison of strain BG2-19 with *S. panayensis* is shown in Fig. 5. The analysis of the phylogenic tree indicated that strain BG2-19 was also closely related to *S. lincolnensis* and *S. capoamus*. Strain BG2-43 gene (451 bp) showed the highest homology (97%) with *Streptoverticillium olivoreticuli* ssp. A comparison of strain BG2-43 with *Streptoverticillium olivoreticuli* ssp. is shown in Fig. 6. Based on the homology value, strain BG2-43 could be a new strain.

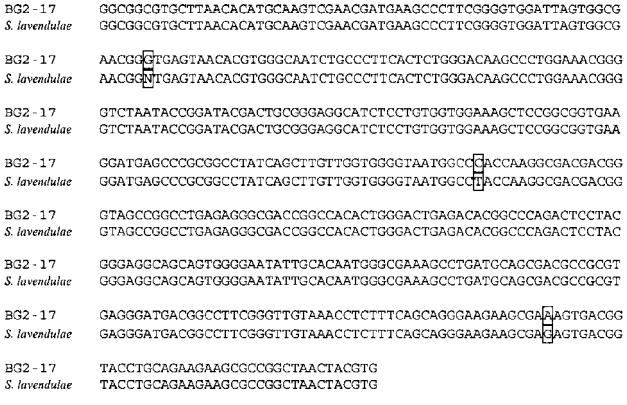

Fig. 4. A comparison of strain BG2-17 with *Streptomyces lavendulae*.

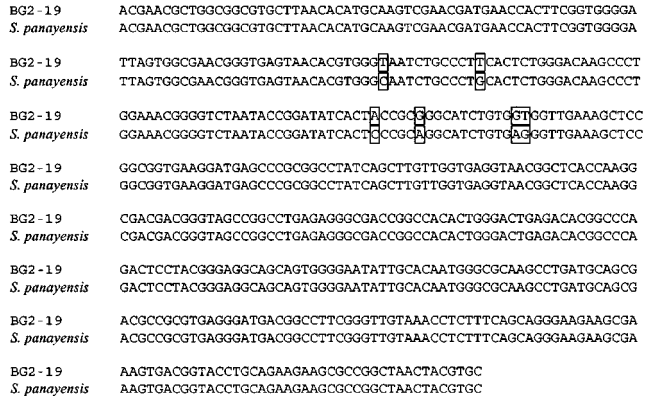

Fig. 5. A comparison of strain BG2-19 with *Streptomyces panayensis.*

Fig. 6. A comparison of strain BG2-43 with *Streptoverticillium olivoreticuli* ssp.

In conclusion, screening tests for *in vivo* antifungal activities against *P. brassicae* revealed three strains, BG2-17, BG2-19, and BG2-43, among forty-eight tested strains to have potent antifungal activities.

Acknowledgment

This work was supported by a grant from Biogreen 21 project in 2001.

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