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Short Communication

Potential of an antagonistic bacterium isolate obtained from *Lentinus lepideus* basidiospores as a biocontrol agent

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The *Burkholderia* sp. isolate 87–11 obtained from basidiospores of *Lentinus lepideus* was antagonistic against several *Pythium* and *Rhizoctonia* isolates. The bacterium was tested against soilborne diseases of five plants caused by *P. aphanidermatum* and *R. solani* by soil and seed application, and its potential as a biocontrol agent is discussed.

Key Words——antagonism; biological control; *Burkholderia* sp.; seed treatment; soil treatment.

For successful biological control of various plant diseases, effective biocontrol agents have been sought and tested in research projects world-wide, because only a few such agents have been used practically.

An antagonistic bacterium was obtained from the bacterial colonies associated with single basidiospores of *Lentinus lepideus* (Fr.: Fr) Fr. fruit bodies collected at Hachioji, Tokyo, Japan in July 1987, showing a marked inhibition (inhibition zone: 28–30 mm broad) against the colony of *L. lepideus* on potato-dextrose agar (PDA) medium (Fig. 1). Therefore, the potential of the bacterium as a biocontrol agent against soilborne diseases was tested, and a part of this work was presented by Watanabe (1991a).

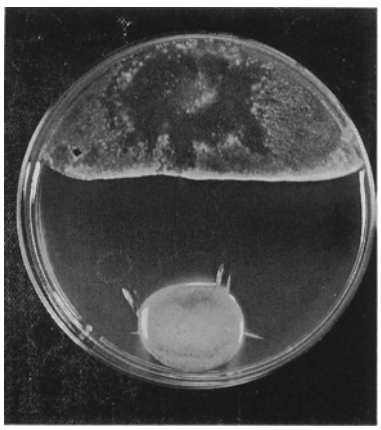

Fig. 1. Marked inhibition zone between colonies of *Lentinus lepideus* and bacterium associated with the basidiospore of *L. lepideus* on PDA in a plate 10 d after isolation.

The antagonistic bacterium (isolate 87–11) was gram-negative, aerobic, straight rod, further characterized by positive reactions in motility, gelatin hydrolysis, catalase reaction, oxidase reaction, and utilization of D-galactose, glucose, inositol and sorbitol, and negative reactions in production of fluorescent pigments and starch hydrolysis. It was found to belong to the genus *Burkholderia* on the basis of phenotypic characteristics and phylogenetic analysis of 16S rRNA gene sequences using CLUSTAL W multiple sequence alignment program (Thompson et al., 1994) (Fig. 2), and to be closely related to *B. gladioli*, *B. cocovenenans*, and *B. vandii*.

Test fungi included several isolates deposited in the Gene Bank, National Institute of Biological Resources, Ministry of Agriculture, Forestry and Fisheries (MAFF) in Tsukuba, Ibaraki, Japan, and the American Type Culture Collection (ATCC) in Manassas, VA, U.S.A. They are one isolate of *Dematophora necatrix* Hartig 84–373 (MAFF 425314) from poplar root collected in Sakaide, Kagawa, Japan; five isolates of *Pythium* spp. including *P. aphanidermatum* (Edson) Fitzpatrick 80–80 (MAFF 305854) obtained from cucumber fruit in Munakata, Fukuoka, Japan and 82–992 from strawberry field soil in Tenri, Nara, Japan, *P. carolinianum* Matthews 85–54 (MAFF 425155, ATCC 66260) isolated from cherry seeds obtained in Hachioji, Tokyo, Japan and *P. periplocum* Drechsler 85–53 (MAFF 425156) from cherry seeds obtained in Hachioji, an unidentified *Pythium* 87–18 from Japanese cedar roots in Nagano, Japan; two isolates of *Rhizoctonia solani* Kühn, 74–333 (MAFF 237780) from scabbed potato obtained in Shizuoka, Japan and 85–60 from cherry seeds from Hachioji.

The bacterium 87–11 was antagonistic against *P.*

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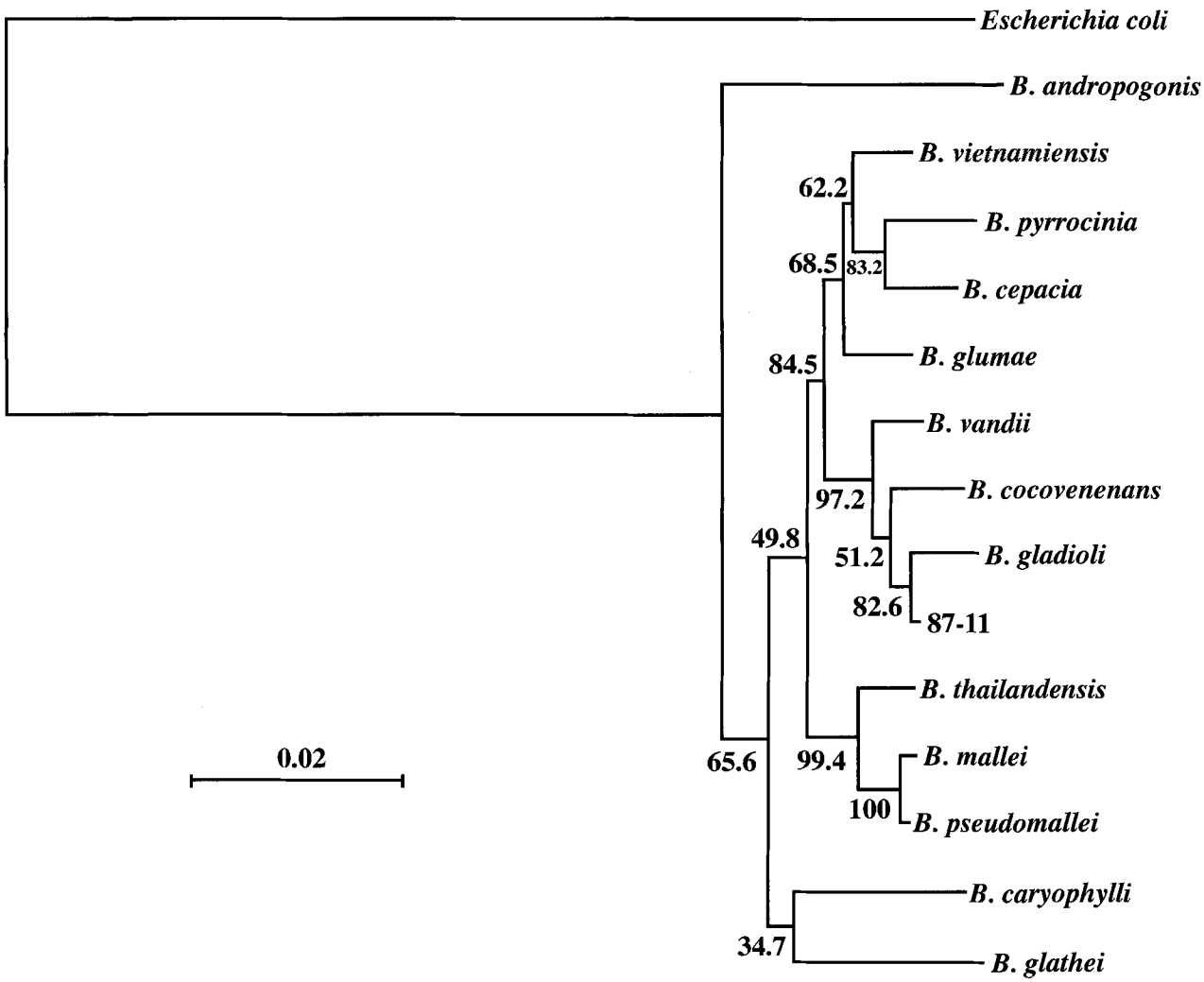

Fig. 2. Phylogenetic analysis of the anagonistic bacterium 87–11 belonging to the genus *Burkholderia* by the molecular analysis of the 16S rRNA gene sequence.

aphanidermatum (isolates 80–80 and 82–992), *P. carolinianum* 85–54, *P. periplocum* 85–53, *Pythium* sp. 87–18, and *R. solani* 74–333, forming 8.6±1.6, 3.6±0.2, 9.6±1.1, 12.0±0.3, 8.0±0.4, and 6.0±0.4 mm broad inhibition zones (means with standard errors) in co-culture with the respective fungi 4 cm apart on PDA in

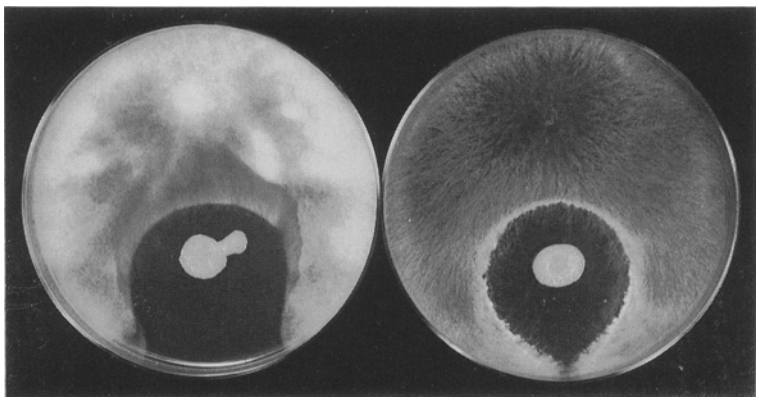

Fig. 3. Inhibition zones formed on PDA between the antagonistic bacterium 87–11 and *Pythium* sp. 87–18 (left) and *Rhizoctonia solani* 74–333 (right) 10 d after inoculation.

plates (Fig. 3). The inhibition zone formation might be related to antibiotic production by this bacterium, because various bacteria have been reported to produce several antibiotics (Homma and Suzui, 1989; Howell and Stipanovic, 1979, 1980).

For biocontrol experiments, seed or soil treatments were conducted following the previous work by Watanabe (1991b). Bacterial suspensions were mostly obtained from 3- to 16-d-old slant cultures (18× 180 mm) with 10 ml of sterilized water.

For seed treatment with bacterium 87–11, seeds were soaked in the bacterial suspension (2.6×10^6–1.3×10^8 cells/ml) for 1, 6, 15, and 20 h and used as treated seeds; and sterilized water was used for untreated controls. After treatment, seeds (10/plate) were immediately sown in the infested soil prepared by covering 7-d-old colonies of the respective pathogens with the autoclaved forest nursery soil (50 ml/plate), watered with 10 ml of tap water and kept moist. For soil treatment, the bacterial suspension (10 ml/plate) was directly poured over the uninfested and infested soil in each plate, and 8 d after treatment, commercial seeds were sown. Each experiment was conducted at least twice in a growth chamber regulated at 26°C for 12 h of light and 20°C for 12 h of darkness. Results were obtained mostly 10 d after sowing for cucumber (*Cucumis sativus* L., cv. Tokiwa-Jibai), turnip (*Brassica campestris* L. rapifera group, cv. Komatsuna-Misugi), radish (*Raphanus sativus* L., cv. Minowase No. 3), and spinach (*Spinacia oleracea* L., cv. Ourai) by checking seedling standing rates, which were determined based on the number of seeds tested. For Japanese black pine (*Pinus thunbergii* Parl.), results were similarly obtained 25 d after sowing.

In the soil artificially infested with *R. solani* 74–333, seedling standing rates (%) of radish and spinach were increased by the treatment with antagonistic *Burkholderia* sp. 87–11, but some spinach and turnip seedlings were stunted by the seed treatment (Table 1). In the soil infested with *R. solani* 85–60, radish and spinach seedlings were healthy after seed treatment, but more severe stunting of spinach occurred after soil treatment. The virulence of *R. solani* 85–60 might have increased synergistically with this bacterium. Japanese black pine was damped off completely by both *R. solani* isolates, and the application of *Burkholderia* sp. 87–11 did not alleviate this effect (Table 1).

In the soil infested with *P. aphanidermatum* (isolates 82–992 and 80–80), the antagonistic bacterium 87–11 was effective for disease control in cucumber seedlings (Fig. 4), but in spinach and other hosts, the application was ineffective or pathogenic, because the host plants tested were stunted. The disease occasionally became more severe in Japanese black pine and turnip following the bacterium application (Table 1.).

Burkholderia sp. 87–11 was also effective against mulberry white root rot disease caused by *Dematophora*

Table 1. Effects of the antagonistic *Burkholderia* sp. 87–11 (=B87) on soilborne diseases of five plants tested caused by *Rhizoctonia solani* and *Pythium aphanidermatum* by soil and seed treatment.

Experiment	Healthy seedling standing rates (%)[a]					
	Radish Soil	Spinach		Turnip Seed	Cucumber Seed	Black pine Seed
		Soil	Seed[b]			
Pathogen: *R. solani* (two isolates: 74–333(=R74), 85–60 (=R85))						
Cont.	60±6	70±6	93±4	95±3	100	68±5
B87	80±4	57±15	83±2	50±6	91±3	66±5
R74	0	0	0	15±3	20±9	0
R85	70±6	93±3	70±17	Nt[c]	Nt	1±1
R74+B87	30±13	23±9	38±6	15±3	33±6	2±1
R85+B87	75±3	37±9	90±2	Nt	Nt	1±1
Pathogen: *P. apanidermatum* (two isolates: 80–80(=P80), 82–992 (=P82))						
Cont.	Nt	Nt	87±9	95±3	100	68±5
B87	Nt	Nt	83±3	50±6	89±2	66±5
P80	Nt	Nt	83±3	Nt	93±5	50±9
P82	Nt	Nt	77±9	85±6	65±13	46±7
P80+B87	Nt	Nt	90±6	Nt	95±3	38±9
P82+B87	Nt	Nt	67±15	50±9	88±4	39±5

a) Healthy seedling standing rates (%, means of 4 or 12 samples with standard errors) of five plants grown in the soil artificially infested with *R. solani* and *P. aphanidermatum* and in the uninfested soil as a control tested using a total of 40 seeds each of radish, spinach, turnip, and 120 seeds each of cucumber and Japanese black pine in the respective experiments.

b) Seeds of spinach and turnip, cucumber, and Japanese black pine soaked in the bacterial suspensions for 6, 15, and 1 h, respectively.

c) Not tested.

Fig. 4. Biocontrol of the damping-off of cucumber seedlings caused by *Rhizoctonia solani* by seed treatment with the antagonistic bacterium 87–11. The seedlings grown on the infested soil (center) were completely collapsed, while those grown in the uninfested control were healthy (left), and two healthy seedlings grew after the seed treatment (right), 10 d after sowing.

necatrix under the greenhouse conditions in preliminary studies, although it was weakly pathogenic to mulberry shoots when suspensions were applied to 1-mo-old seedlings transplanted in the diseased soil (data not shown).

Burkholderia gladioli (=*Pseudomonas gladioli*) (Arie et al., 1987) and other *Burkholderia* species including *B. cepacia* (=*Pseudomonas cepacia*) (Homma et. al., 1989; Kawamoto and Lorbeer, 1976) and *B. fluorescens* (=*P. fluorescens*) (Howell and Stipanovic, 1979, 1980) are known to be effective against soilborne diseases caused by *Fusarium*, *Pythium*, and *Rhizoctonia* spp., and the antagonistic bacterium 87–11 was also effective in certain combinations of host plants and pathogens in this study. However, further work is necessary for practical usage of this bacterium as a biocontrol agent, because it caused stunting in a few plants tested, and synergistic effects were noted in virulence with a few weak pathogens.

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