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# Screening for *In Vitro* Antifungal Activity of Soil Bacteria Against Plant Pathogens

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**Antifungal bacteria for biological control of plant diseases or production of novel antibiotics to plant pathogens were isolated in 1997 from various soils of Ansung, Chunan, Koyang, and Paju in Korea. Sixty-four bacterial strains pre-screened from approximately 1,400 strains were tested on V-8 juice agar against eight plant pathogenic fungi using *in vitro* bioassay technique for inhibition of mycelial growth. Test pathogens were *Alternaria mali*, *Colletotrichum gloeosporioides*, *C. orbiculare*, *Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *lycopersici*, *Magnaporthe grisea*, *Phytophthora capsici*, and *Rhizoctonia solani*. A wide range of antifungal activity of bacterial strains was found against the pathogenic fungi, and strain RC-B77 showed the best antifungal activity. Correlation analysis between inhibition of each fungus and mean inhibition of all eight fungi by 64 bacterial strains revealed that *C. gloeosporioides* would be best appropriate for detecting bacterial strains producing antibiotics with potential as biocontrol agents for plant pathogens.**

**KEYWORDS: Antifungal activity, *Colletotrichum gloeosporioides*, Fungi, Plant pathogen, Screening, Soil bacteria**

Concern about the negative effects of synthetic fungicides in agricultural production has led to the search for biocontrol agents and naturally occurring substances with antifungal activity from fungi, bacteria, algae, and higher plants. Both microorganisms and their metabolites have been investigated to minimize pesticide usage and deleterious effects on environment (Hebber and Lumsden, 1999). Soil has thousands of microbial species including approximately 30,000 bacteria species and 1,500,000 fungal species (Lee, 1994). The isolation and selection of microorganisms from the soil are the first step in the search for novel antibiotics and/or biologically active microorganisms effective against plant pathogens. Screening for effective candidates usually involves time-consuming procedures. Therefore, mycelial growth assays with target fungi have been frequently used for the purpose (Boyyetchko, 1999; Broadbent *et al.*, 1971). In the present study, we isolated bacterial strains from various soils, using an inhibition assay of mycelial growth, to search for bacterial strains producing novel antibiotics which might also be antagonistic to plant pathogens.

Bacterial strains were obtained in 1997 from various field soils of Ansung, Chunan, Koyang, and Paju in Korea. Soil samples were taken using an open-end soil probe (20 cm deep, 2.5 cm in diameter) (UDY Corporation, Fort Collines, CO, USA), put into polyethylene bags, and stored in an ice chest. Each soil sample (25 g) was put into a 500-ml Erlenmeyer flask containing 250 ml sterile distilled water. Flasks were shaken on a rotary shaker (150 rpm) for 30 min, and dilutions of the samples were made before plating on tryptic soy agar (Difco, Detroit, MI, USA) amended with 50 mg $l^{-1}$ cycloheximide. Bacterial strains were isolated from the cultures after incubation for 2~3 days at 28°C.

Antifungal activity of approximately 1,400 bacterial strains was pre-tested on V-8 juice agar (pH 6.4) against plant pathogenic fungi, *Alternaria mali*, *Colletotrichum gloeosporioides*, *C. orbiculare*, *Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *lycopersici*, *Magnaporthe grisea*, *Phytophthora capsici*, and *Rhizoctonia solani*. From this prescreening, 64 bacterial strains (Table 1) were selected for further examination. The 64 bacterial strains or sterile water (control) were streaked in a line down the centers of V-8 juice agar and incubated for 24 h. Mycelial disks (5 mm in diameter) from the actively growing 7-day-old cultures of the fungi were placed on both sides of the V-8 juice plate. Inhibition of mycelial growth of each fungus by bacterial strains was measured when fungal mycelia in the water controls reached the center of the plate.

Experiments were conducted twice with three replications, in which one replication indicated the mean of the two measurements of each test plate. Statistical analyses were conducted with pooled data from repeated experiments using the Statistical Analysis System (SAS Institute, 1988). Analysis of variance was conducted using the general linear models procedure (LSD) and means were separated using the least significance difference. Relationships between inhibition length of each fungus and mean inhibition length of eight fungi by 64 bacterial strains were examined using the correlation analysis.

A wide range of antifungal activity of bacterial strains against plant pathogenic fungi was found in the test of mycelial growth of fungi on V-8 juice agar (Fig. 1 and Table 1). Among 64 strains tested, strain RC-B77 showed the highest antifungal activity to plant pathogenic fungi while strains LS-B80, OA-B15, and OC-B18 did the lowest.

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**Table 1.** Antifungal activity of 64 bacterial strains to eight plant pathogenic fungi on V-8 juice agar

| Bacterial strain | Inhibition of mycelial growth (mm)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | AM[b] | CG | CO | FOC | FOL | MG | PC | RS | Mean |
| RC-B77 | 23.5[c] | 14.2 | 26.7 | 10.4 | 10.3 | 18.3 | 15.0 | 19.7 | 17.3 |
| GC-B27 | 15.8 | 15.8 | 21.8 | 15.8 | 15.7 | 23.0 | 18.8 | 7.6 | 16.8 |
| RC-B33 | 21.1 | 13.1 | 26.4 | 10.5 | 9.7 | 18.5 | 15.0 | 20.7 | 16.8 |
| GC-B33 | 16.1 | 15.4 | 20.8 | 13.6 | 15.1 | 21.8 | 18.7 | 9.9 | 16.4 |
| GK-B26 | 15.6 | 15.5 | 21.1 | 15.8 | 14.9 | 22.1 | 18.8 | 7.1 | 16.4 |
| MM-B25 | 16.7 | 14.3 | 22.0 | 14.0 | 13.0 | 21.2 | 20.5 | 9.1 | 16.4 |
| GC-B07 | 15.4 | 14.7 | 20.4 | 15.8 | 13.6 | 22.9 | 18.9 | 8.2 | 16.2 |
| GC-B32 | 14.5 | 15.7 | 20.6 | 14.9 | 15.3 | 21.4 | 19.3 | 8.2 | 16.2 |
| RC-B39 | 21.6 | 14.8 | 25.3 | 10.5 | 10.5 | 16.9 | 10.5 | 17.3 | 15.9 |
| GC-B35 | 15.1 | 14.3 | 20.7 | 14.0 | 14.2 | 21.7 | 19.3 | 6.8 | 15.8 |
| GK-B21 | 15.3 | 15.0 | 21.2 | 14.8 | 13.6 | 22.0 | 17.1 | 7.3 | 15.8 |
| MW-B02 | 16.5 | 15.8 | 19.7 | 13.6 | 13.6 | 19.9 | 20.1 | 6.9 | 15.8 |
| MW-B19 | 14.7 | 14.2 | 22.0 | 13.5 | 13.4 | 21.1 | 19.1 | 9.3 | 15.8 |
| RC-B40 | 20.8 | 13.5 | 25.1 | 11.3 | 9.0 | 18.0 | 13.0 | 17.0 | 15.8 |
| GK-B29 | 14.4 | 14.9 | 20.1 | 14.7 | 14.3 | 21.5 | 18.0 | 7.2 | 15.7 |
| GK-B28 | 14.6 | 14.9 | 20.6 | 14.3 | 13.8 | 20.8 | 18.7 | 7.7 | 15.5 |
| OA-B36 | 15.2 | 13.8 | 21.8 | 13.2 | 11.9 | 21.6 | 18.8 | 8.0 | 15.5 |
| RK-B26 | 15.6 | 13.4 | 21.8 | 13.2 | 12.6 | 21.5 | 19.1 | 7.0 | 15.5 |
| GK-B24 | 14.3 | 14.4 | 20.9 | 13.3 | 12.1 | 20.5 | 19.1 | 5.3 | 15.0 |
| OA-B22 | 13.7 | 13.6 | 20.5 | 13.4 | 10.8 | 21.4 | 19.5 | 6.9 | 15.0 |
| GK-B09 | 13.6 | 14.4 | 21.1 | 12.4 | 11.7 | 20.6 | 18.9 | 5.9 | 14.8 |
| GK-B15 | 14.2 | 14.0 | 20.1 | 12.5 | 13.4 | 19.7 | 16.6 | 9.9 | 14.8 |
| GK-B18 | 13.7 | 14.4 | 19.0 | 13.1 | 12.9 | 19.6 | 17.5 | 6.2 | 14.5 |
| GK-B25 | 11.8 | 12.8 | 18.2 | 13.6 | 13.3 | 20.6 | 16.5 | 7.3 | 14.3 |
| RC-B78 | 14.1 | 15.2 | 22.1 | 11.7 | 10.3 | 25.6 | 4.7 | 9.7 | 14.2 |
| GC-B23 | 13.1 | 13.3 | 17.0 | 9.9 | 12.7 | 20.3 | 15.0 | 9.4 | 13.9 |
| MM-B16 | 20.6 | 11.0 | 23.1 | 6.5 | 5.4 | 15.1 | 12.2 | 17.0 | 13.9 |
| OA-B65 | 13.8 | 13.3 | 19.4 | 12.1 | 11.5 | 18.2 | 15.7 | 6.6 | 13.8 |
| VK-B14 | 12.4 | 12.1 | 20.7 | 11.8 | 11.3 | 18.9 | 16.3 | 7.4 | 13.8 |
| LS-B01 | 12.8 | 11.1 | 19.6 | 10.7 | 9.8 | 19.7 | 17.9 | 7.2 | 13.5 |
| OA-B26 | 12.6 | 12.3 | 18.6 | 11.9 | 9.9 | 19.8 | 16.0 | 6.4 | 13.5 |
| PK-B09 | 12.3 | 13.4 | 18.9 | 11.5 | 11.3 | 16.9 | 15.7 | 7.3 | 13.4 |
| LS-B42 | 13.9 | 11.3 | 18.8 | 11.4 | 10.2 | 18.2 | 16.6 | 6.4 | 13.3 |
| RC-B38 | 14.3 | 9.3 | 20.1 | 11.2 | 6.0 | 11.5 | 14.4 | 15.8 | 12.8 |
| RC-B65 | 12.8 | 12.5 | 19.4 | 8.5 | 9.0 | 24.1 | 3.7 | 7.1 | 12.3 |
| GC-B26 | 11.2 | 11.4 | 16.8 | 9.4 | 7.8 | 16.7 | 15.0 | 5.1 | 11.8 |
| LS-B70 | 8.4 | 5.9 | 18.9 | 3.5 | 2.1 | 21.8 | 3.1 | 2.3 | 8.3 |
| OC-B70 | 7.4 | 5.8 | 12.2 | 4.3 | 4.7 | 14.6 | 1.2 | 2.3 | 6.6 |
| MM-B22 | 5.7 | 4.4 | 6.9 | 8.5 | 7.9 | 1.7 | 8.7 | 3.1 | 5.9 |
| MM-B03 | 4.4 | 3.5 | 7.0 | 8.1 | 7.4 | 2.6 | 8.5 | 4.4 | 5.7 |
| MM-B01 | 4.8 | 4.2 | 8.2 | 6.4 | 7.3 | 2.9 | 8.4 | 2.5 | 5.6 |
| MM-B20 | 4.0 | 3.2 | 7.7 | 8.5 | 7.3 | 3.0 | 7.3 | 3.0 | 5.6 |
| GC-B17 | 5.5 | 5.7 | 9.3 | 3.2 | 2.4 | 8.2 | 0.7 | 1.4 | 4.6 |
| MW-B24 | 5.7 | 5.1 | 5.8 | 3.1 | 2.6 | 5.7 | 0.8 | 2.0 | 3.9 |
| MW-B18 | 5.0 | 5.4 | 6.6 | 2.8 | 3.1 | 5.9 | 0.0 | 1.6 | 3.8 |
| GC-B19 | 5.0 | 4.3 | 8.3 | 1.2 | 1.1 | 6.9 | 0.4 | 0.9 | 3.4 |
| PK-B26 | 4.5 | 4.4 | 5.3 | 2.9 | 1.3 | 6.3 | 0.0 | 1.3 | 3.3 |
| GC-B28 | 2.8 | 4.1 | 10.6 | 0.2 | 0.6 | 1.8 | 1.2 | 0.0 | 2.7 |
| PK-B14 | 3.5 | 3.9 | 9.9 | 0.9 | 0.9 | 1.6 | 0.8 | 0.0 | 2.7 |
| OA-B03 | 2.2 | 2.5 | 9.8 | 1.4 | 0.0 | 2.3 | 2.9 | 0.0 | 2.6 |
| OA-B37 | 1.8 | 3.4 | 10.5 | 1.0 | 1.0 | 0.0 | 2.9 | 0.0 | 2.6 |
| GC-B24 | 2.9 | 3.0 | 9.5 | 0.6 | 0.4 | 1.8 | 1.2 | 0.0 | 2.4 |
| LS-B03 | 2.6 | 2.7 | 9.0 | 0.7 | 0.6 | 1.8 | 0.9 | 0.0 | 2.3 |
| RC-B41 | 1.5 | 1.6 | 9.6 | 0.6 | 0.2 | 0.7 | 3.0 | 0.0 | 2.1 |
| MW-B10 | 1.2 | 0.9 | 6.5 | 0.6 | 5.7 | 0.8 | 0.0 | 0.0 | 2.0 |
| RC-B64 | 1.7 | 1.4 | 7.3 | 1.2 | 0.4 | 1.3 | 1.8 | 1.0 | 2.0 |
| RC-B37 | 0.3 | 0.9 | 6.0 | 0.4 | 0.7 | 3.5 | 1.6 | 0.9 | 1.8 |
| VC-B11 | 1.1 | 4.1 | 5.1 | 1.6 | 0.7 | 2.0 | 0.0 | 0.0 | 1.8 |
| RK-B41 | 0.9 | 2.8 | 5.3 | 1.4 | 1.1 | 0.2 | 0.0 | 0.0 | 1.4 |
| LS-B81 | 0.5 | 1.1 | 5.7 | 0.0 | 0.5 | 1.1 | 1.3 | 0.0 | 1.3 |
| MW-B15 | 0.7 | 1.9 | 4.0 | 1.1 | 1.1 | 0.0 | 1.1 | 0.0 | 1.2 |
| LS-B80 | 0.3 | 2.3 | 2.3 | 0.4 | 0.3 | 1.1 | 0.7 | 0.0 | 0.9 |
| OA-B15 | 0.0 | 0.4 | 5.0 | 1.3 | 0.1 | 0.5 | 0.0 | 0.0 | 0.9 |
| OC-B18 | 0.3 | 1.8 | 3.3 | 1.2 | 0.4 | 0.0 | 0.0 | 0.0 | 0.9 |
| $LSD_{0.05}$ | 3.0 | 2.1 | 3.6 | 2.0 | 2.2 | 2.8 | 2.2 | 2.3 | 1.9 |

[a]Inhibition of mycelial growth was determined when mycelia in water controls reached the center of the V-8 juice agar plate.
[b]AM = *Alternaria mali*, CG = *Colletotrichum gloeosporioides*, CO = *C. orbiculare*, FOC = *Fusaium oxysporum* f. sp. *cucumerinum*, FOL = *F. oxysporum* f. sp. *lycopersici*, MG = *Magnaporthe grisea*, PC = *Phytophthora capsici*, and RS = *Rhizoctonia solani*.
[c]Values are means of six replications from two experiments.

In addition, RC-B77 for *A. mali* and *C. orbiculare*, MW-B02 for *C. gloeosporioides*, GC-B27 for *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *lycopersici*, RC-B78 for *M. grisea*, MM-B25 for *P. capsici*, and RC-B33 for *R. solani* showed the greatest antifungal activity, respectively (Fig. 1 and Table 1). Thus the inhibition assay of mycelial growth successfully identified bacterial strains antifungal against plant pathogenic fungi as has been widely used in many researches (Broadbent *et al.*, 1971; Chin-A-Woeng *et al.*, 1998; Mazzola *et al.*, 1995; Trejo-Estrada *et al.*, 1998). In addition, antifungal bacteria were found to have similar suppression effects on mycelial growths of nearly all test fungi. These results are well supported by similar observations of Rodriquez and Pfender (1997) that a *Pseudomonas fluorescens* strain producing antifungal metabolites inhibited mycelial growth of *Pyrenophora tritici-repentis*, *Drechslera poae*, and *Sclerotinia homoeocarpa*.

When correlation between inhibition length of each fungus and mean inhibition length of eight fungi by 64 bacterial strains was analyzed, *C. gloeosporioides* showed the highest correlation coefficient at $P = 0.001$ and *R. solani* did the lowest (Table 2). This implies that *C. gloeosporioides* would be best appropriate for detecting bacterial strains producing antibiotics or biological control agents. Other fungi except *R. solani* are also available for the purpose since they showed high levels of correlation coefficients. Further research with these antifungal strains screened from this study will be needed for searching novel antibiotics and/or determining biological control efficacy in the greenhouse and field as well as identifying strains.

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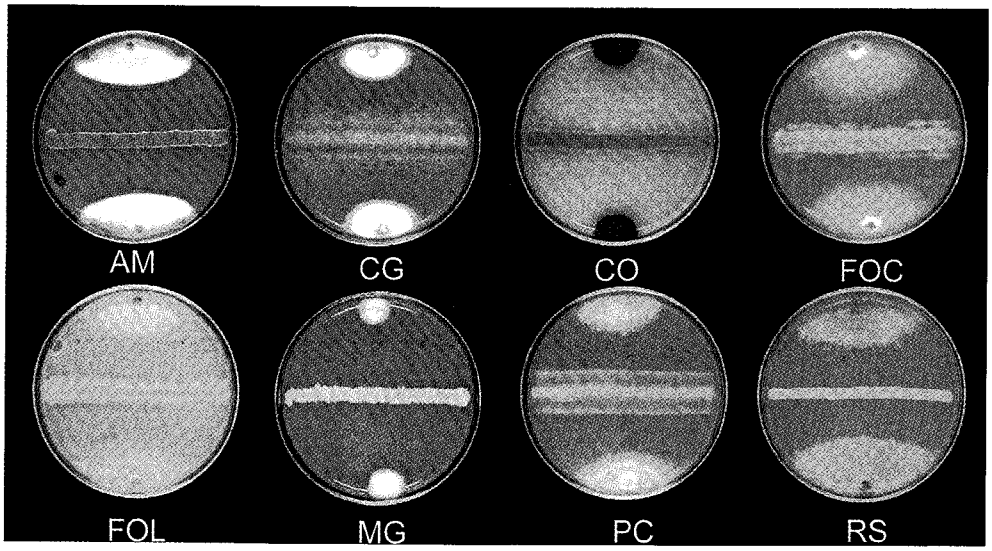


**Fig. 1.** Inhibition of mycelial growth of plant pathogenic fungi, *Alternaria mali* (AM), *Colletotrichum gloeosporioides* (CG), *C. orbiculare* (CO), *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), *F. oxysporum* f. sp. *lycopersici* (FOL), *Magnaporthe grisea* (MG), *Phytophthora capsici* (PC), and *Rhizoctonia solani* (RS) by bacterial strains, RC-B77, MW-B02, RC-B77, GC-B27, GC-B27, RC-B78, MM-B25, and RC-B33, respectively. Photographs for bacterial strains were taken when fungal mycelia in water controls reached the centers of the V-8 juice agar plates. Bacterial strains or water (control) were streaked in a line down the centers of the V-8 juice agar plates 24 hrs before inoculation of plant pathogenic fungi.

**Table 2.** Correlation coefficients between inhibition length of each plant pathogenic fungus and mean inhibition length of eight fungi by 64 bacterial strains on V-8 juice agar

| Plant pathogenic fungus | Correlation coefficient[a] |
|---|---|
| *Alternaria mali* | 0.946 |
| *Colletotrichum gloeosporioides* | 0.961 |
| *C. orbiculare* | 0.937 |
| *Fusarium oxysporum* f. sp. *cucumerinum* | 0.938 |
| *F. oxysporum* f. sp. *lycopersici* | 0.916 |
| *Magnaporthe grisea* | 0.939 |
| *Phytophthora capsici* | 0.912 |
| *Rhizoctonia solani* | 0.795 |

[a]Correlation coefficients are significantly different at $P = 0.0001$. This analysis was conducted using pooled data from two experiments with three replications each.

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