

## Research Note

### PSEUDOMONAS CEPACIA, A POTENTIAL BIOFUNGICIDE FOR ROOT ROT PATHOGENS OF BEANS<sup>1, 2</sup>

Concern about the risk that pesticides pose to the environment and human health has increased the need for more knowledge about biological control methods in crop production. The biological control of pathogens can be accomplished through the use of plant resistance, cultural practices and antagonistic microorganisms. Although biological control will not replace the use of pesticides completely, it will increase in importance as integrated control programs become more commonplace. Biocontrol is also an important component in low-input sustainable agricultural systems.

Fungal species of *Macrophomina*, *Rhizoctonia*, *Fusarium*, *Sclerotium* and *Pythium* have been reported in Latin America<sup>3</sup> and Puerto Rico<sup>4</sup> as causing root rot in common bean (*Phaseolus vulgaris*). There is a lack of information in the region about the adaptation of alternative control measures to reduce yield losses to those fungal root rot pathogens. The use of biofungicides for those fungi is still in its infancy and much work is needed to achieve better control and consistent field response.

A strain of *Pseudomonas cepacia* that strongly inhibits the growth of *Macrophomina phaseolina* in vitro was previously identified in the Biotechnology

and Nitrogen Fixation (BNF) Laboratory at the University of Puerto Rico, Mayagüez Campus.<sup>5</sup> The objectives of this study were to confirm the inhibitory growth of *M. phaseolina* by *P. cepacia* strain UPR 5C and to test, in vitro, the interaction of *P. cepacia* with other root rot fungal pathogens.

*Macrophomina phaseolina* (PRMp2), *Rhizoctonia solani* (Rs1), *Sclerotium rolfsii* (Sr1), *Pythium aphanidermatum* (Pa1) and *Fusarium solani* (Fs1) were isolated from diseased common bean grown in northwestern Puerto Rico. *P. cepacia* strain UPR 5C was obtained from the BNF Collection at the University of Puerto Rico-Mayagüez. Fungi were grown on potato dextrose agar (PDA) and UPR 5C on tryptone yeast (TY) media.<sup>6</sup>

Two in vitro methods, streak plate and double-layer, were used to measure the antibiosis of UPR 5C towards the fungi. The fungal radial growth was measured in millimeters and the percentage of inhibition was obtained by using the formula: % inhibition =  $1 - [F + B/F_1] \times 100$ ; in which  $F+B$  is the radial growth of the fungus plus bacteria (B); and  $F_1$  is the radial growth of the fungus alone.

The experiment design for each in vitro test was a factorial arrangement of six fungi species and two treatments (with and without *P. cepacia*) in a randomized

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<sup>3</sup>Abawi, G. S., 1989. Root rots. In: Bean Production Problems in the Tropics. 2nd. Edition. Ed. Schwartz, H. H. and Pastor-Corrales, M. A. CIAT, Cali, Colombia.

<sup>4</sup>Echávez-Badel, R. and J. S. Beaver, 1987. Dry bean genotypes and *Macrophomina phaseolina* (Tassi) Goid. in inoculated and noninoculated field plots. *J. Agric. Univ. P.R.* 71: 385-390.

<sup>5</sup>Perdomo, F., E. C. Schröder and R. Echávez-Badel, 1990. Antagonistic microorganisms towards *Macrophomina phaseolina* in vitro. *Phytopathology* 80: 1050.

<sup>6</sup>Beringer, J. E., 1974. R factor transfer in *Rhizobium leguminosarum*. *J. Gen. Microbiol.* 84: 188-189.

complete block with five replications. Data obtained were subjected to analysis of variance and differences between treatment means were tested by an LSD.

In the streak plate method, *P. cepacia* was streaked on one half of a TY agar (pH 6.8) and incubated for 5 days at 28° C. One disk each of actively growing PRMp2, Rs1, Sr1, Pa1 and Fs1 3-day-old cultures were cut and placed on the half of the TY plate 5 cm from the bacterial streak. After incubating the plates at 28° C for 5 days, the radial growth rates and percentage of inhibition were determined.

For the double-layer method, tempered TY agar was added to 1 ml of bacterial suspension on a Petri dish and distributed throughout the agar by gently swirling the plate. After incubation for 3 days at 28° C, a second layer of 10 ml TY agar was poured over the first layer. Three hours later, one 5 mm disk of each fungus was placed on the center of each plate and incubated at 28° C for 5 days.

Results with the streak plate methods show that strain UPR 5C effectively inhibited the growth of all fungi, but less on *P. aphanidermatum* and *F. solani* (table 1). The inhibition could be due to antibiotic production, competition for nutrients, production of volatile inhibitors, or siderophore production. Howell and Stipanovic<sup>7</sup> reported that *P. fluorescens* produces the antibiotics pyrrolnitrine and pyoluteorin that inhibited the growth of *R. solani*. Lambert et al.<sup>8</sup> and Janisiewicz and Roitman<sup>9</sup> observed that pyrrolnitrine isolated from *P. cepacia* inhibited the growth of several fungi in maize and fruits. Homma et al.<sup>10</sup> reported that pyrrolnitrine and other antibiotics isolated from *P. cepacia* inhibited growth, in vitro, of *Pyricularia oryzae*, *R. solani* and *Verticillium dahliae*.

Results from the double-layer method indicate that *P. cepacia* UPR 5C completely inhibited the growth of all fungi used in this study, excluding *F. solani*. Several other researchers have also reported

TABLE 1.—*In vitro* inhibitory effect of *Pseudomonas cepacia* (Pc) (UPR 5C) towards root rot fungi of beans (*Phaseolus vulgaris*)

Pathogenic fungi	Percentage inhibition	
	Streak plate	Double-layer
<i>Sclerotium rolfsii</i> (Sr1)	100 <sup>1</sup>	100
<i>Rhizoctonia solani</i> (Rs1)	95	100
<i>Macrophomina phaseolina</i> (PRMp2)	93	100
<i>Pythium aphanidermatum</i> (Pa1)	67	100
<i>Fusarium solani</i> (Fs1)	41	60
LSD (0.05)	3.4	—

<sup>1</sup>Average of four replications.

<sup>7</sup>Howell, C. R., and R. D. Stipanovic, 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopathology* 70: 712-715.

<sup>8</sup>Lambert, B., F. Leyns, L. V. Rooyen, F. Gosselé, Y. Papon and J. Swings, 1987. Rhizobacteria of maize and their antifungal activities. *Appl. Environ. Microbiol.* 53: 1866-1871.

<sup>9</sup>Janisiewicz, W. J. and J. Roitman, 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. *Phytopathology* 78: 1697-1700.

<sup>10</sup>Homma, Y., Z. Sato, F. Hirayama, K. Konno, H. Shirahama and T. Suzui, 1989. Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soil-borne plant pathogens. *Soil Biol. Biochem.* 21: 723-728.

that *P. cepacia* completely inhibits the growth of some plant pathogenic fungi and partially inhibits others.<sup>5, 7, 11</sup>

In both, the streak plate and double-layer methods, *Macrophomina phaseolina* (PRMp2) was strongly inhibited by UPR 5C. These results confirm the earlier findings of Perdomo et al.<sup>5</sup> The biocontrol mechanism of UPR 5C has not been identified conclusively. It appears that antibiosis and nutrient competition may play important roles. Correlation of in vitro antagonisms and in vivo protection in the field has been reported.<sup>12, 13, 14</sup> Inoculation with *P. cepacia* strain UPR 5C could

be used as a seed inoculant to protect beans from root-rot pathogens. The effect seems to be strain-specific since other strains of *P. cepacia* did not show strong inhibitory effects (Schröder, unpublished results).

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<sup>11</sup>Jayaswal, R. K., M. A. Fernández and R. G. Schroeder III, 1990. Isolation and characterization of a *Pseudomonas* strain that restricts growth of various phytopathogenic fungi. *Appl. Environ. Microbiol.* 56: 1053-1058.

<sup>12</sup>Aldrich, J. and R. Baker, 1970. Biological control of *Fusarium roseum* f. sp. *dianthi* by *Bacillus subtilis*. *Plant Dis. Rep.* 54: 446-448.

<sup>13</sup>Broadbent, P., K. F. Baker, N. Franks and J. Holland, 1977. Effect of *Bacillus* spp. on growth of seedlings in steamed and in non-treated soil. *Phytopathology* 67: 1027-1034.

<sup>14</sup>Filippi, C., G. Bagnoli and G. Picci, 1985. Effetto antagonista di batteri terricoli su *Fusarium oxysporum* Schlecht f. sp. *dianthi*. II Effetto antimicotico ad ampio spettro di un batterio del terreno nei riguardi di specie fitopatogene, opportinistiche e saprofite. *L'Agr. It.* 1/2: 27-40.