INHIBITION MECHANISMS OF **Macrophomina phaseolina** by **Burkholderia cepacia** UPR 5C

Rodrigo Campo, R. Echávez-Badel, and E.C. Schröder. Departments of Crop Protection and Agronomy & Soils. Univ. of Puerto Rico, Mayagüez Campus, Mayagüez, P.R. 00681-5000.

Charcoal root rot caused by **Macrophomina phaseolina** has become an important disease of common bean in Puerto Rico and the Dominican Republic. Recent results indicated that **M. phaseolina** isolates obtained in Puerto Rico (PRMp1, PRMp2) and the Dominican Republic (RDMp1, RDMp2) differ in virulence, and some common bean varieties exhibited slight or severe symptoms under greenhouse conditions (**2,4**).

In vitro inhibition studies conducted at the Biotechnology and Nitrogen Fixation (BNF) Laboratory of the University of Puerto Rico, Mayagüez, suggested that a strain of **Burkholderia cepacia**, previously **Pseudomonas cepacia** (**12**), strongly inhibited the growth of **M. phaseolina** (**10,11**). **B. cepacia** UPR 5C strain has been characterized by laboratory standard methods and identified by surveying the metabolics properties by the Biolog's technology (**9**).

The production of antifungal agents by bacteria, especially by bacteria associated with plants, offers great potential to control plant pathogens. The mechanisms involved include production of antibiotics, siderophores, competition for nutrients and acidic conditions. The objective of this work is to determine the possible inhibition mechanisms of **M. phaseolina** by **B. cepacia** UPR 5C strain.

Antibiotic and gas production were tested as potential inhibition mechanisms of **B. cepacia**. The inclusion and streak plate methods and tryptose-yeast (TY) agar medium were used to measure the antibiosis of **B. cepacia**. Treatments were replicated 3 times and results analyzed by analysis of variance. Differences between treatments and control were obtained by L.S.D. test.

Antibiotic production: Results of treating cells with heat (autoclave) using the inclusion method showed that only living cells and cells treated with chloroform significantly inhibited fungal growth of PRMp2, RDMp1 and RDMp2 isolates (**Table 1**), indicating the production of a heat-sensitive antifungal substance. Results confirm those reported by various authors (**5,7,8**), who isolated and identified several antibiotics in different strains of **P. cepacia**.

Gas production: **B. cepacia** was grown with **M. phaseolina** in divided Petri dishes, streaking UPR 5C on one side of the dish and placing a 5 mm fungus disk on the other side. Results indicate that UPR 5C was able to reduce significantly the growth of **M. phaseolina** isolates by 81% (**Table 2**). Although we did not collect and identify the volatile compounds produced, they probably included ammonia, since the growth medium TY is rich in N. These findings are similar to those reported by DePasquale and Montville (**3**) and Howell et al. (**6**). Baligh et al. (**1**), identified ammonia among the volatile compounds produced by another strain of **P. cepacia**. Characterization and identification of antifungal substances are contemplated.

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Table 1. Antibiosis of **Macrophomina phaseolina** (PRMp2, RDMp1, RDMp2 radial growth by **Burkholderia cepacia** UPR 5C.

Treatment	Inhibition [1]
Living cells	4.00 a**
Killed cells with chloroform	4.00 a
Filtrate	1.00 b
Autoclaved cells	1.00 b
Control (TY + chloroform)	1.00 b
L.S.D. (P=0.01)	0.195
C.V. (%)	6.92

[1] Inhibition scale: 1= no inhibition; 4= total inhibition ** Mean values in column followed by the same letter do not differ significantly at the 1% probability level.

Table 2. Inhibitory effect of UPR 5C gases towards **Macrophomina phaseolina** (PRMp2 isolate) radial growth.

Treatment	Radial Growth (mm)	Inhibition (%)
UPR 5C + PRMp2	5.7 a**	81.0
Control (fungus alone)	30.0 b	0.0
L.S.D. (P=0.01)	2.79	
C.V. (%)	6.30	

** Mean values in column followed by the same letter do not differ significantly at the 1% probability level.