

# ANTAGONISTIC ACTIVITY OF SPECIES OF *PSEUDOMONAS* AGAINST *BOTRYODIPLODIA THEOBROMAE* PAT. IN CULTURE

E. C. WOKOMA

(Received 11 July 2000; Revision accepted 5 September 2000)

## ABSTRACT

Fourteen strains of *Pseudomonas* that produced yellowish-green diffusible pigments on King's medium B were screened for ability to inhibit growth of *Botryodiplodia theobromae* on Yeast Extract Sucrose Mineral salts (YESM) broth. Mycelial disks of *B. theobromae* were introduced into Petri dishes containing YESM broth 48 h after the medium was inoculated with bacterial cells. These were compared with standard biocontrol pseudomonads, *Pseudomonas fluorescens* Pf-5, and *P. putida* W4P5. Mycelial dry weight determined 72 h after growing the fungus in YESM-bacterial culture revealed that the growth of the fungus was significantly reduced by over 50 % in the presence of some of the bacterial strains. Three strains, classified as strong antagonists, were comparatively antagonistic as *P. fluorescens* Pf-5. Six strains were moderate antagonists, with their antagonistic ability not being significantly different from that of *P. putida* W4P5. Two strains were weak antagonists. Three strains did not show any antagonistic action against the growth of *B. theobromae* in culture.

**Key words:** Antagonistic, *Botryodiplodia theobromae*, standard biocontrol *Pseudomonas*.

## INTRODUCTION

*Botryodiplodia theobromae* Pat., is frequently isolated from diseased tissues in the tropics. The pathogen is commonly associated with rots of several crops in the field and in storage (Arinze, 1985; Lutchmeah; 1994). Benomyl, mancozeb and chlorothalonil are used to control the fungus in the field and storage. The scarcity, high cost of purchase, and improper application of fungicides by farmers in Nigeria are some of the numerous problems associated with the successful control of the pathogen. There is the identified need to seek for alternative, cheap; effective and safe ways of reducing the activity of the pathogen for crop improvement, particularly ways that are environmental friendly with no adverse effects on crops, domestic animals and man. The use of natural enemies as biocontrol agents against fungal pathogens of plants has been widely reported (Howell and Stipanovic 1980, Loper 1988, Kloepper et al. 1988). The objective of this study was to test the potential ability of some strains of *Pseudomonas* isolated from tropical crops to inhibit the growth of *B. theobromae*. The ability of a bacterial strain to inhibit growth of the fungus is indicative of the possible role of the strain as a biocontrol agent (Cook and Baker 1983, Xu and Gross 1986).

## MATERIALS AND METHODS

Fourteen strains of *Pseudomonas species* that produced pigments on King's medium B (KMB) plates were isolated from the root segments, or

root washings of corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill), waterleaf (*Talinum triangulare* L.), plantain (*Musa paradisiaca* L.), banana (*Musa* sp.), cassava (*Manihot esculenta* Crantz), pawpaw (*Carica papaya* L.); and fluted pumpkin (*Telfaria occidentalis* Hooker Fil.). After repeated streaking to obtain cultures, the strains were identified as described in the Bergey's Manual (Buchanan and Gibbons 1974) and routinely stored at 4°C on KMB slants. *P. fluorescens* Pf-5 and *P. putida* W4P5 were included as standards. Both strains were received as lyophilized stocks from Dr. D. C. Gross of the Department of Plant Pathology, Washington State University, Pullman, Washington State. After reviving the lyophilized cultures in Nutrient broth (Oxoid), pure colonies were obtained by repeated streaking on KMB plates and also maintained at 4°C on KMB slants. The fungus *B. theobromae* was isolated from rotted yam (*Dioscorea rotundata* Poir) tuber on Oxoid potato dextrose agar (PDA) plates acidified with one to two drops of 85 % lactic acid. Pure cultures were maintained on acidified PDA plates.

The medium used for the study was Yeast Extract Mineral Salts (YESM) broth. It contained 0.9 g K<sub>2</sub>HPO<sub>4</sub>, 0.2 g KCl, 0.2 g CaCl<sub>2</sub>, 0.2 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.002 g FeSO<sub>4</sub>, 0.002 g MnSO<sub>4</sub>, 0.1 g Yeast Extract, 1.0 g NH<sub>4</sub>NO<sub>3</sub>, and 5.0 g sucrose, in 1 litre deionised water (Chao 1990). The compounded medium was autoclaved for 15 minutes at 121°C, 1.03 Kg Cm<sup>-2</sup>. The procedure used to test for interaction between the bacterial

strains and the fungus were a modification of the method described by Chao (1990). In this study, bacterial cells were used instead of culture filtrates. Colonies of a 24 hr culture of each strain was suspended in sterile deionized water, after which one ml of  $2.0 \times 10^7$  colony forming units per ml suspension was placed in glass Petri dishes (100 x 15 mm) containing 20 ml YESM broth. Each Petri dish containing the bacteria-YESM mixture was incubated for 48 hr at ambient temperature (29°C), in the dark, for the growth of the bacteria. Petri dishes containing YESM broth alone served as control. After a 48-hr incubation period, three mycelial disks (5 mm diameter), cut from the edge of a 3-day-old culture of *B. theobromae* were placed in each Petri dish including the control. All Petri dishes were further incubated in the dark at ambient temperature for 72 hr. During incubation, the dishes were frequently agitated for aeration of the medium and suspension of the bacterial cells. At the end of the second incubation period, the mycelium from each dish was collected by passing the broth culture through a pre-weighed Whatman No. 1 qualitative filter paper, rinsed twice with deionised water and dried in a 60°C oven, overnight. After drying, the filter paper with the dried mycelium was reweighed.

Antagonistic activity of each strain of *Pseudomonas* was determined by comparing the weight of the dried mycelium from the fungus-bacteria culture with mycelium of the fungus grown alone in the YESM broth medium. A completely randomized design with 17 treatments replicated three times was used. The experiment was repeated once and similar results obtained. Data on mycelial dry weight from both trials were combined and subjected to analysis of variance (ANOVA) to determine significant differences. The Duncan's Multiple Range Test (DMRT) determined mean separation at 5% level of probability.

## RESULTS AND DISCUSSION

The antagonistic activity of the *Pseudomonas* strains, expressed as reduction in dry weight of *B. theobromae* on YESM broth is shown in Figure 1. Comparison of the dry weights of the fungal mycelia indicated that test strains PA-FP, PP-WC2, and PA-PP were the most antagonistic, performing better than one of the standard strains, *P. putida* W4P5. Of the two standard strains, Pf-5 was significantly more antagonistic to the growth of *B. theobromae*, reducing growth of the fungus by over 50%. No significant differences in antagonism were observed among the following strains: PP-WW3, PF-SC1, PF-WT2, PA-ST2, PA-B, and PP-WA. Three strains PP-WW1, PA-WW2 and PF-B were non-antagonists. There was no significant difference between the dry weight of *B.*

*theobromae* mycelium grown with the cells of these three bacterial strains and the dry weight of the fungus grown alone in the YESM broth medium. Three levels of antagonists were identified in the study viz.:

- (a) Strong antagonists - PA-FP, PP-WC2, and PA-PP isolated from fluted pumpkin, corn and pawpaw.
- (b) Moderate antagonists - strains PP-WW3, PF-SC1, PF-WT2, PA-ST2; PA-B, and PP-WA isolated from waterleaf, corn, tomato, and banana.
- (c) Weak antagonists - strains PA-WC1, and PP-CA isolated from corn and cassava.
- (d) Non-antagonists - strains PP-WW1, PA-WW2, and PF-B isolated from waterleaf and banana.

Differences in antagonism were evident among the 16 strains of *Pseudomonas* tested. The *in vitro* screening tests carried out indicated that potential antagonists were resident in the rhizosphere of the crops used in the study. Although these crops were obtained from the fields of farmers in Rivers State, they are likely to occur in other parts of the Niger Delta. *P. fluorescens* strain Pf-5 and *P. putida* strain W4P5 which were originally isolated from field crops in the United States of America, served as model antagonists. They significantly reduced the growth of the fungus. *P. fluorescens* strain Pf-5, is known for its ability to antagonise some fungal pathogens *in vitro* (Kloepper 1990, Kraus and Loper 1990). Three of the strains tested were similar to strain Pf-5 in activity while six other strains were also similar to strain W4P5 in activity.

Though bacterial inhibition of plant pathogens *in vitro* is often used for the screening of potential biocontrol agents, some findings have shown that strong inhibition on Petri plates does not always parallel strong inhibition *in vivo* and vice versa. Janisiewicz (1988) reported that some organisms that strongly inhibited *Botrytis cinerea* Pers. Ex. Fr. and *Penicillium expansum* Link Ex Thom did not display strong inhibition on apple fruits. Lesion development on apples also depended on the qualitative relationship between the antagonists and the pathogen propagules. Chuang and Yang (1993) reported that one bacterial isolate out of every four tested *in vitro*

reduced the number of banana lesions on banana infected by *Collectotrichum musae* under natural conditions. In this study only a few strains were not very effective antagonists against *B. theobromae* *in vitro*. *In vivo* studies would further confirm their biocontrol potentials. *Botryodiplodia theobromae* is a widespread pathogen in the tropics. Its ability to abundantly produce viable propagules as well as survive in plant debris for long periods makes this pathogen difficult to control (Ekundayo and Daniels 1973, Aderiye

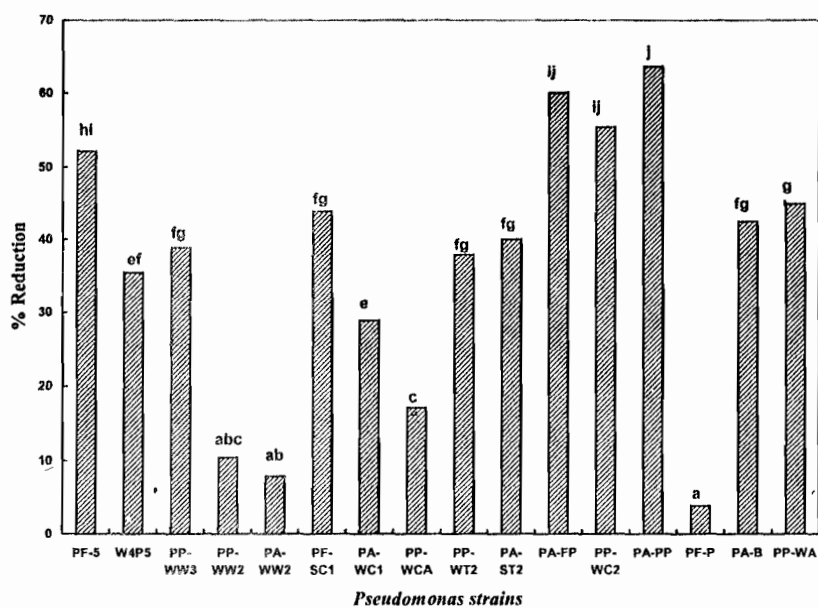


Figure 1. Reduction in mycelial dry weight of *Botryodiplodia theobromae* by strains of *Pseudomonas*.

1983). Ekundayo (1984) reported that 6-methylpurine inhibited pycnidial production by the fungus. The present study shows that rhizosphere bacteria can inhibit the mycelium of *B. theobromae*. Research has shown that biocontrol will reduce the need for chemical pesticides (Whitaker 1993). The undesirable effects of pesticides on human health and the environment have created much global concern. The need for pesticide education and training for tropical smallholders to ensure that pesticides are used safely effectively, and appropriately is a high priority for the crop protection industry and should be a high priority for governments of tropical countries. In the absence of 'safe-use' initiatives, and detailed research on environmental impact of pesticides, it is needful to adopt cultural practices that will enhance the colonisation of roots by beneficial rhizoflora. Since biological control is based on the interaction of beneficial and pathogenic organisms in the ecosystem, cultural practices incorporating the use of compost and organic manure would enhance the rapid multiplication of rhizobacteria and other soilborne antagonists. The need for further study and search for beneficial rhizoflora has been established.

#### ACKNOWLEDGEMENTS

I am grateful to D. C. Gross for providing the lyophilised cultures of *P. fluorescens* Pf-5 and *P. putida* W4P5 used in the study. The Technical assistance of P. Omobor is acknowledged. I am thankful to T. V. Otokunefor for critical review of the manuscript.

#### REFERENCES

- Aderiyi B. I., 1983. Survival of *Botryodiplodia theobromae* Pat. during unfavourable conditions. M. Sc. Thesis, University of Ife, Nigeria (unpublished).
- Arinze A. E., 1985. Action of polygalacturonase and cellulase enzymes on yam (*Dioscorea* spp) and sweet potato (*Ipomoea batatas*) tissues. *Phytopath. Z.* 114 : 234-242.
- Buchanan R. E., and Gibbons N. E., 1974. *Bergey's Manual of Determinative Bacteriology* (8<sup>th</sup> edition). William and Wilkins Company, Baltimore.
- Chao W. L., 1990. Antagonistic activity of *Rhizobium* species against beneficial and plant pathogenic fungi. *Letters in Applied Microbiology* 10: 213-215.
- Chuang T. Y., and Yang H. R., 1993. Biological control of banana anthracnose. *Plant Pathology Bulletin* 2: 71-77.
- Cook R. J., and Baker K. F., 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society, St Paul, Minneapolis. 539pp.
- Ekundayo C. A., 1984. Inhibition of pycnidial production of *Lasiodiplodia theobromae* by 6-methylpurine. *Trans. Br. Mycol. Soc.* 82: 346-347.

- Ekundayo J. A., and Daniels T. M., 1973. Cassava rot and its control. *Trans. Br. Mycol. Soc.* 61: 27-33.
- Howells C. R., and Stipanovic R. D., 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. *Phytopath.* 70: 712-715.
- Janisiewicz W. J., 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. *Phytopath.* 78: 194-198.
- Kloepper J. W., Hume D. J., Scher F. M., Singleton C., Tipping B., Laliberte M., Frauley K., Kutchaw T., Simpson C., Lifshitz R., Zaleska I., and Lee, L., 1988. Plant growth-promoting rhizobacteria on canola (rapeseed). *Plant Disease* 72: 42-46.
- Kloepper J. W., 1990. Development of an *in vivo* assay for selecting potential rhizobacterial biological control agents against *Rhizoctonia solani* on cotton. In : Abstract Book, The Second International Workshop on Plant Growth Promoting Rhizobacteria, Interlaken, Switzerland. October 14-19, 1990, pg. 73.
- Kraus J. and Loper J. E., 1990. Biocontrol of pythium damping-off of cotton and cucumber by *Pseudomonas fluorescens* Pf-5: Mechanistic Studies. In: Abstract Book, The Second International Workshop on Plant Growth Promoting Rhizobacteria, Interlaken, Switzerland. October 14-19, 1990, pg. 56.
- Loper J. E., 1988. Role of fluorescent siderophore production in the biological control of *Pythium ultimum* by *Pseudomonas fluorescens* strain. *Phytopath.* 78: 166-172.
- Lutchmeah R. S., 1994. Postharvest deterioration of papaya in Mauritius. *Proceeding 1<sup>st</sup> Conference on African Mycology*, Mauritius, June 10-15, 1990.
- Whitaker M. J., 1993. The challenge of pesticide education and training for smallholders. *Int. J. Pest Management* 39: 117-125.
- Xu, G-W, and Gross, D. C., 1986 Field evaluation of the interactions among fluorescent pseudomonads, *Erwinia caratovora* and potato yields. *Phytopath.* 75: 423-430.