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INHIBITION OF SELECTED FUNGI BY BACTERIAL ISOLATES FROM *TRIPSACUM DACTYLOIDES* L.

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Key words

Allelopathy Caryopsis Fungal inhibition Pseudomonas *Tripsacum dactyloides*

Summary

A bacterium (*Pseudomonas* sp.), which was repeatedly isolated from the caryopses of the grass, *Tripsacum dactyloides*, inhibited the growth of selected species of fungi, including *Penicillium chrysogenum*, *Rhizopus stolonifer*, and *Trichoderma viride*. This inhibition may prevent fungal decomposition of caryopses of *T. dactyloides*.

Introduction

During a germination study of *Tripsacum dactyloides* (eastern gamagrass, 2N = 72), the moistened filter paper on which caryopses were placed became covered with fungal growth. However, a zone of inhibition frequently surrounded each caryopsis. Only when embryos lost viability or geminated did fungal mycelia grow over the caryopses. Some caryopses that contained viable embryos, as determined by eventual germination, permitted no mycelial development for 12 months.

Because several investigators have reported various forms of microbial inhibition by seeds of vascular plants[5,6,7,9], we assumed initially that the plant was producing an allelopathic[9] substance, which was causing the inhibition. Because some caryopses of *T. dactyloides* apparently remain on the soil for 18 months or longer before germination occurs, resistance to decomposition through fungal inhibition seemed to be a plausible adaptation.

Methods and results

To study the nature of the inhibition, the following experiments were performed. In the first experiment we aseptically removed ten *T. dactyloides* caryopses from their glume-rachis structure and incubated them in 10 ml of sterile distilled water at 30°C for 48 hours.

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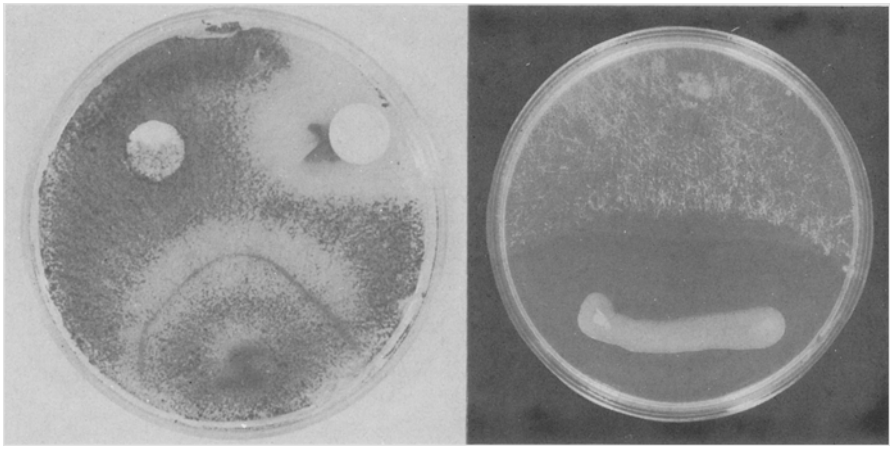
Plant and Soil 56, 149–152 (1980). 0032-079X/80/0561-0149$00.60.



A B

Plate 1

A Representative bioassay in 150 × 15 mm petri dish. Experimental disc (*right*) inhibited the fungus *T. viride*, and the fungus overgrew the control disc (*left*).

B Inhibition of *Rhizopus stolonifer* by the bacterium (*Pseudomonas* sp.) that was isolated from *T. dactyloides*.

Some of this liquid was drawn onto sterile, 13 mm Schleicher and Schnell # 740-E assay paper discs by capillary action. These discs and control discs, prepared from sterile distilled water, were placed near the edges of Petri dishes containing 2% corn meal agar. The Petri dishes were inoculated at the opposite edge with *Trichoderma viride* (ILL 74), a common soil fungus involved in decomposition, and incubated at 30°C for 48 hours.

Most experimental discs strongly inhibited *T. viride*, but fungal growth quickly covered control discs (Plate 1A). Also, bacterial growth invariably appeared around discs that showed inhibition. If caryopses, which had been incubated in distilled water for 48 hours, were used in place of the discs in the bioassay test, a zone of inhibition developed only if bacteria grew around the caryopses. Soaking the caryopses for 48 hours did not affect seed viability.

When the bacteria around the discs and caryopses were cultured on Difco Tryptic Soy Agar supplemented with 0.2% Yeast Extract, at least eight different bacteria could be distinguished by colony morphology, Gram-reaction, and microscopic appearance. We isolated a Gram-negative, aerobic bacterium, approximately 1 × 3 μm with a single polar flagellum that has been identified as a *Pseudomonas* sp. This was the only bacterium, which inhibited *T. viride*. Separate experiments have shown that the inhibiting bacterium can be 'reisolated' routinely.

A second experiment was carried out to determine if the inhibition was caused by the bacterium, an allelopathic material produced by the caryopses, or an interaction between plant and bacterium. Ten caryopses of *T. dactyloides* were incubated in each of three test tubes containing 10 ml of sterile distilled water for 48 hours at 30°C in the dark. The liquid from each tube was passed through a Millipore HAWP filter (0.45 μm pore size) and

divided into two parts. Half of the filtrate from each tube was used in the bioassay as described in the previous experiment. The second half received bacteria and was incubated for 48 hours at 30°C, and duplicate bioassays were performed using these bacteria.

In the final experiment, the Pseudomonas was introduced into Petri dishes containing 2% corn meal agar and previously inoculated with selected species of fungi. The bacterium was introduced by either streaking cells across the agar surface or by placing paper discs, previously soaked in a Nutrient Broth culture, on the agar surface. In addition to *T. viride*, we tested the bacterium's ability to inhibit *Rhizopus stolonifer* (NRRL 1477), *Penicillium chrysogenum* (ISU 42), *Fusarium moniliforme* (ATCC 32426), and *Alternaria* sp. (ISU 1648).

In the second experiment, discs soaked in filtrate produced no inhibition. Discs soaked in the bacterial suspension produced strong inhibition. Strong inhibition also occurred (in the last experiment) when the bacterium was either streaked across the agar surface (Plate 1B) or introduced via paper assay discs moistened with a Nutrient Broth culture.

We found that the bacterium was inhibitory to *T. viride*, *R. stolonifer*, and *P. chrysogenum*, but not to *Alternaria* sp. or *F. moniliforme*.

Discussion

It is difficult to interpret the significance of the occurrence of this fungal-inhibiting bacterium (*Pseudomonas* sp.) on caryopses of *T. dactyloides*. Nevertheless, there are relatively few reports describing this kind of interaction between seeds and microorganisms. The occurrence of fungal species of *Chaetomium* on oats protects seedlings from the pathogen *Helminthosporium victoriae* and inhibits other fungi and bacteria[3,10,11]. Reports of bacterial inhibition of fungi are less common[8]. *Bacillus subtilis* occurring in soil was shown to protect barley seedlings from the fungus *Helminthosporium sativum*[1,2]. However, this is the first report of a bacterium associated with caryopses that appears to prevent their fungal decomposition.

Many questions remain concerning the nature of the inhibition that we report here. The bacterium and plant may be interacting in a mutualistic manner: the plant embryo may provide a metabolic product(s) for the bacterium, which inhibits some fungi. The success of the bacterium in inhibiting fungi in the absence of the caryopsis could be due to the culture medium providing the necessary growth factor(s) normally produced by the plant. It is also important to determine if the bacterium is able to inhibit fungi that occur naturally on the caryopses and are capable of causing its degradation. Studies currently in progress are considering this question.

The possibility of a new antifungal agent of bacterial origin is encouraging. Only since 1959 has antifungal chemotherapy been possible, and even now the number of useful antifungal drugs is limited[4,12].

Acknowledgements

We appreciate the helpful suggestions of Drs. Herman Brockman and Brian Wilkinson, Department of Biological Sciences, Illinois State University, in preparation of the manuscript.

Received 17 May 1979.

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