Inhibition of Bacterial Growth by Extracts from Potato Tissues

J. C. Zalewski and Luis Sequeira

Research Assistant and Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706, respectively.

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ABSTRACT

Ethanolic extracts of tuber, stem, and leaf tissues of *Solanum phureja* and *S. tuberosum* 'Russet Burbank' inhibited growth of the wilt-inducing bacterium, *Pseudomonas solanacearum*. Inhibitory activity was consistently higher (two- to three-fold) in extracts from wilt-resistant clones of *S. phureja* than in those from the wilt-susceptible *S. tuberosum*. Inhibitory activity was reduced in extracts of *S. phureja* plants grown at low light intensity 6,046 lx (600 ft-c), a condition known to reduce resistance to the bacterium. In liquid culture, low concentrations of the inhibitor increased the lag period and decreased the logarithmic phase growth rate of *P. solanacearum*. Concentrations that were bactericidal to *P. solanacearum* did not inhibit growth of *Erwinia atroseptica* or *E. carotovora*. Partial purification yielded an active fraction with ultraviolet absorption maximum at 264 nm and chemical properties differing from those of previously described antimicrobial compounds from potato tubers.

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Additional key words: Pseudomonas solanacearum, antibiotics, disease resistance.

Bacterial wilt caused by *Pseudomonas solanacearum* E. F. Sm. is one of the most serious diseases of potato in tropical and subtropical areas. The bacterium multiplies in the xylem and spreads systemically to most plant parts, including the tubers. Infected tubers show typical discoloration of the vascular ring but decay remains localized in this area (3). In his early studies on potato wilt, Smith (9) reported that the bacterium did not grow well on raw potato slices; we have confirmed these observations. A possible explanation for this phenomenon is that potato tubers contain substances which inhibit growth of the pathogen. In addition, it seemed possible that the concentration and distribution of these substances might differ in potatoes resistant or susceptible to the disease.

High levels of resistance to bacterial wilt have not been found in *Solanum tuberosum* L. (6, 7), but have been reported in certain clones of *S. phureja* Juz. & Buk. (10). Sequeira & Rowe (8) demonstrated that resistance from *S. phureja* can be transferred to *S. tuberosum*. The purpose of this paper is to report on antibacterial substances which appear to be present at higher concentrations in extracts from *S. phureja* than in those from *S. tuberosum*.

To prepare crude extracts, approximately 100 g of potato tuber or stem tissues were cut in small pieces, added to 400 ml of boiling 95% ethanol and comminuted in a Waring Blendor for 5 min after cooling. The suspension was filtered and the tissue was extracted with 95% ethanol in a Soxhlet apparatus for 18 hr. The ethanolic extracts were combined and evaporated to dryness under reduced pressure. The residue was suspended in 75 ml distilled water and centrifuged at 27,000 *g* for 15 min. The supernatant fluid was evaporated to dryness under reduced pressure and the residue was dissolved in 5 ml 0.05 M potassium phosphate buffer, pH 7.0. For bioassay, this crude extract was sterilized by filtration through a Millipore filter (pore size 0.45 μ).

The biological activity of crude extracts was determined by growth inhibition of *P. solanacearum* on agar and in broth cultures. In the first method, 0.1 ml of extract was placed in each of four wells cut in each plate of casamino acids-peptone-glucose (CPG) agar seeded with isolate K-60. The plates were incubated at 30 C for 48 hr and the areas of the zones of inhibition surrounding each well were determined. In the second method, 1 ml of a bacterial suspension (2×10^9 cells/ml) was added to a 10-ml test tube containing 1 ml each of diluted sterile extract, 0.2 M potassium phosphate buffer (pH 7.0), and CPG liquid medium. Distilled water was added instead of extract to control tubes. The tubes were placed in a water bath shaker at 30 C. Optical density was measured (at 600 nm) every 2-4 hr with a Bausch & Lomb Spectronic 20 colorimeter. After 10 hr of growth, inhibition was determined from the difference in absorbance between tubes containing culture extracts and the controls.

All crude extracts from *S. phureja* tubers (10 g fresh wt equivalent/ml) produced large zones of inhibition surrounding wells in plates seeded with isolate K-60. Inhibition zone area, rather than diam, gave a substantially straight line when plotted against the logarithm of the concentration of extract. Other isolates of *P. solanacearum* (B-1, S-225, and S-206) were equally as sensitive as K-60 to inhibitors in the extracts. On the other hand, soft-rotting bacteria, such as *Erwinia carotovora* (Kelman SR40) and *E. atroseptica* (Kelman SR8) (which cause rapid breakdown of potato tuber tissues) appeared to be stimulated, rather than inhibited, by equivalent concentrations of the extract in the plate assay. Similarly, in the standard liquid medium assay, dilutions at 1:10, 1:20, and 1:40 of a stem extract (20 g fresh wt/ml) stimulated growth of the two *Erwinia* spp., but markedly inhibited growth of *P. solanacearum* except at 1:40 (Fig. 1-A).

The crude extract from *S. phureja* stems was bacteriostatic at low concentrations (e.g., 0.5 g fresh wt equivalent/ml) and bactericidal at high concentrations (e.g., 1.0 g/ml) in the liquid medium assay (Fig. 1-B).

To determine the relative amounts and distribution of inhibitory compounds in resistant and susceptible potatoes, extracts were prepared from: (i) freshly harvested tubers of *S. tuberosum* 'Russet

Burbank' and of *S. phureja*, clone 1386.26; and (ii) stems from Russet Burbank and *S. phureja* (clones 1386.26 and 1386.9) plants grown from sprouted tubers for 4 weeks in a growth chamber at 21 C, 21,500 lx (2,000 ft-c), and a 14-hr photoperiod. Extracts were assayed by the agar plate diffusion method.

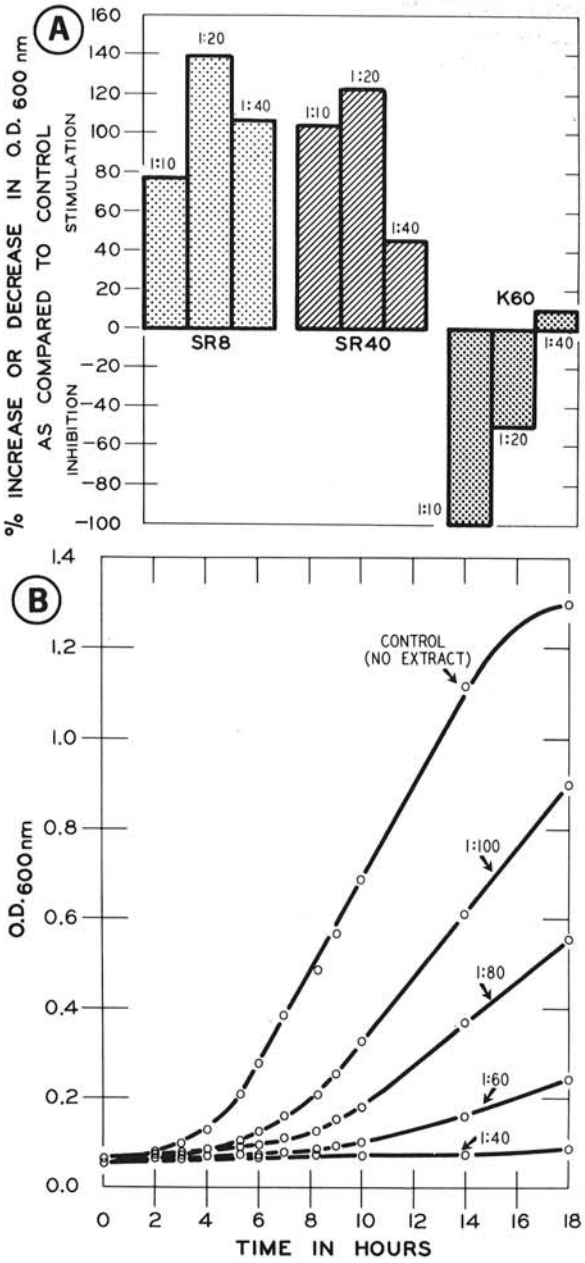

Fig. 1. A) Differential effect of a crude extract from *Solanum phureja* (1386.26) on growth of *Erwinia atroseptica* (SR8), *E. carotovora* (SR40), and *Pseudomonas solanacearum* (K-60), after 10 hr in liquid casamino acids-peptone-glucose culture. Designated dilutions of an extract containing the equivalent of 20 g fresh wt/ml; **B)** Inhibitory effect of crude extract from *S. phureja* (1386.26) stems on growth of *Pseudomonas solanacearum* (K-60) in liquid culture. Dilutions of extract containing the equivalent of 40 g fresh wt/ml.

Inhibition of bacterial growth was obtained with extracts from all tissues, but amounts of inhibition were greater with extracts of *S. phureja* clones than with those of Russet Burbank. For instance, extracts containing 100 g fresh wt equivalent each of periderm or tuber flesh (cortex, vascular cylinder, peri-medullary zone, and central pith) per ml gave average zones of inhibition of 486 and 409 mm^2 for clone 1386.26, and 164 and 20 mm^2 for Russet Burbank, respectively.

Similarly, inhibitory activity was consistently higher in extracts of *S. phureja* stems than in those from Russet Burbank (Table 1). Based on dilution end points, inhibitory activity of *S. phureja* extracts was at least two- to three-fold greater than that of Russet Burbank. Extracts from stem tissue of a third *S. phureja* clone (1339.28) were assayed in a later experiment and were found to inhibit *P. solanacearum* at concentrations approximately equal to those of 1386.26 and 1386.9.

S. phureja clones 1386.9 and 1339.28 are more susceptible than 1386.26 to *P. solanacearum* (8), but are more resistant to *P. solanacearum* than *S. tuberosum* Russet Burbank (Sequeira & Rowe, *unpublished*). A sufficient number of clones of *S. phureja* and *S. tuberosum* have not been examined to allow correlations of inhibitor content with degree of resistance to *P. solanacearum*. Differences in resistance between *S. phureja* clones probably cannot be attributed to a single inhibitory compound, since it has been shown that resistance in *S. phureja* is highly specific for certain strains of the pathogen (8), and these strains are equally sensitive to the inhibitory fraction described herein.

Since exposure to low light intensity is known to reduce resistance of *S. phureja* plants to *P. solanacearum* (8), 6-week-old plants of clone 1386.26 were held for 1 week at either 19,380 lx (1,800 ft-c) or 6,460 lx (600 ft-c) and 14-hr photoperiod in a growth room at 21 C, and then 50 g samples of stem

TABLE 1. Inhibition of *Pseudomonas solanacearum* (K-60) by a crude extract from stem tissues of (resistant) *Solanum phureja* clones 1386.26, 1386.9 and (susceptible) *S. tuberosum* 'Russet Burbank'

	Area (mm^2) of inhibition zone after 48 hr incubation at 30 C[a]		
	Solanum phureja		
Dilution	Clone 1386.26	Clone 1386.9	Russet Burbank
1:1	80	62	9
1:2	47	40	0
1:3	20	20	0
1:4	0	0	0
1:5	0	0	0

[a] Each figure is the average of two replications. Each well contained a 0.1 ml aliquot of the designated dilution of an extract containing the equivalent of 10 g fresh wt tissue/ml.

tissues from each group were extracted and assayed on plates as before. Inhibitory activity in extracts containing 10 g fresh wt equivalent/ml from plants held under low light (dilution end point 1:2) was approximately 25% lower than that in extracts from plants exposed to high light intensity (dilution end point 1:3).

The inhibitory fraction was located at R_F 0.10 - 0.25 after descending paper chromatography of crude extracts with butanol-acetic acid-water 4:1:5, v/v). This fraction had an UV absorption maximum at 264 nm, did not react with reagents for alkaloids, and was insoluble in diethyl ether, chloroform, ethyl acetate and other organic solvents. These properties suggest that the inhibitor(s) reported here differs from previously described antimicrobial substances obtained from potato tissues (1, 2, 4, 5, 10, 11, 12). Attempts are being made to further characterize this inhibitor.

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