# Effect of Halo Toxin-Containing Filtrates of Pseudomonas phaseolicola on the Growth of Bean Callus Tissue

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ABSTRACT

Growth of bean stem-callus tissue was inhibited 77%, 41%, and 10% when 10% (v/v) culture filtrates of *Pseudomonas phaseolicola, P. syringae,* and *P. morsprunorum,* respectively, were added to the callus tissue culture medium. Only *P. phaseolicola* culture filtrates induced halos on bean leaves. *P. phaseolicola* filtrates diluted 1:10 with water induced no halos. Callus tissue grown in a medium containing 10% (v/v) *P. phaseolicola* culture filtrate contained more total N, less soluble protein, and a much higher level of ornithine than callus tissue grown in control filtrate. Similarities between the physiological effects of toxin-containing filtrates of *P. phaseolicola* on bean callus and on green leaf tissue suggest the feasibility of employing callus tissue to study the mode of toxin action. Phytopathology 60:1065-1067.

*Additional key words: Phaseolus vulgaris,* callus growth inhibition, amino compounds in callus.

Bean (*Phaseolus vulgaris* L.) halo blight, incited by *Pseudomonas phaseolicola* (Burk.) Dows., is characterized by the formation of chlorotic halos around infection foci and the subsequent development of systemic chlorosis. Recent studies on host-parasite relationships in bean halo blight indicate that a bacterial toxin is involved in the production of these symptoms (3, 6). Ornithine accumulation, one of the physiological responses characteristic of tissues affected by the halo toxin, is greater in stems than in leaves or petioles of infected plants (8). Growth of bean stem-callus tissue is inhibited in media containing culture filtrates of *P. phaseolicola* (2, 9). Only culture filtrates from toxin-producing strains of *P. phaseolicola* possessed this property, suggesting that callus growth inhibition is associated with the halo toxin. We now report additional evidence that inhibition of callus tissue growth is due to the toxin produced by the halo blight organism.

MATERIALS AND METHODS.—Callus was established from stem pieces of *P. vulgaris* 'Manitou' light red kidney. Stock cultures were maintained on White's medium (11) supplemented with 2,4-dichlorophenoxy-acetic acid (2,4-D, 1 ppm) and coconut milk (10% v/v). Culture filtrates of *P. phaseolicola* (*Pp*), *P. morsprunorum* (*Pmp*), and *P. syringae* (*Ps*) were prepared by growing the bacteria in a simple, defined medium (1.0 g $NH_4H_2PO_4$, 0.2 g KCl, 0.2 g $MgSO_4 \cdot 7H_2O$, and 10 g dextrose/liter, pH 7.1) for 12 days at 17 C on a reciprocal shaker. Filtrates were centrifuged and sterilized as previously described (2). All filtrates were assayed for halo-inducing ability on primary leaves of Charlevoix dark red and Manitou light red kidney bean plants by the needle-prick method (3).

The effects of bacterial filtrates on stem-callus growth was studied by adding 10% v/v of each filtrate to a basal medium (BM) composed of modified White's medium containing sucrose (3%), inositol (25 ppm), 2,4-D (1 ppm), yeast extract (1,000 ppm), and 0.8% agar. Control media were prepared by adding 10% v/v bacterial culture medium to BM.

All media were adjusted to pH 5.8 prior to autoclaving. Approximately 100 mg fresh wt of actively-growing callus were transferred to the culture media. All experiments were conducted in diffuse light at 22 ± 1 C and 10-20% relative humidity. Suspension cultures, in which the agar was omitted from the BM, were incubated on a gyro-rotary shaker at 100 shakes/min. Dry wt were determined on tissues dried 48 hr in a 60 C oven.

The effect of *Pp* filtrate on total N, soluble protein, and ribose nucleic acid (RNA) levels of callus tissue was determined on tissue grown for 14 days in suspension culture. The oven-dried callus was powdered in a glass tissue grinder, and aqueous solutions were prepared and centrifuged. RNA and soluble protein were determined in the supernatant by the orcinol (5) and Lowry (4) methods, respectively. Total N was determined on powdered tissue using the micro-Kjeldahl method (1).

Free amino acids and ammonia were extracted from callus grown in *Pp* and control filtrates for 35 days using the method described by Patel & Walker (6). Duplicate samples of each extract were analyzed on a Spinco amino acid analyzer, model 120.

RESULTS.—*Pp* filtrate induced halos 8-10 mm in diam on primary leaves of dark red kidney beans within 2 days after needle-prick application; no halos were formed when *Ps, Pmp,* and control culture filtrates were assayed in a similar manner. Dilution of *Pp* filtrate 1:10 with distilled water eliminated the halo-inducing ability.

*Pp* filtrate added to the BM at 10% v/v (1:10 dilution with BM) reduced callus tissue growth by about 77% on a dry wt basis during a 35-day incubation period (Table 1, Fig. 1, 2). Filtrate of *Pmp,* which is not a pathogen of bean, had no significant effect on

TABLE 1. The effect of control and culture filtrates (10% v/v) of *Pseudomonas phaseolicola* (*Pp*), *P. morsprunorum* (*Pmp*), and *P. syringae* (*Ps*), on the growth of Manitou bean callus tissue after 35 days[a]

| Treatment | Callus growth mg dry wt/tube |
|---|---|
| Control filtrate | 41.4 ± 4.0 |
| *Pp* filtrate | 13.5 ± 6.2 |
| *Pmp* filtrate | 37.1 ± 6.4 |
| *Ps* filtrate | 24.5 ± 4.1 |

[a] Each treatment was replicated eight times.

callus growth. Filtrate of *Ps*, a pathogen of bean, reduced callus growth by about 41%. This inhibition may have been due partly to the presence of syringomycin, a biocide produced by this organism in culture (10).

Following a growth period of 14 days, callus tissue consistently contained less soluble protein and more total N when grown in *Pp* filtrate than in control filtrate (Table 2). RNA was not appreciably altered by the *Pp* filtrate.

Callus tissue grown for 14 days in *Pp* filtrate contained, on a fresh wt basis, about 55 times the ornithine, 2 times the alanine, histidine, and lysine, and 1.5 times the methionine and serine as callus tissue grown in control filtrate (Table 3). Ammonia was the only amino compound to decrease in callus grown in the *Pp* filtrate.

DISCUSSION.—Several lines of evidence indicate that the growth inhibition response of bean callus tissue to *Pp* filtrate in the medium is due to the presence of the "halo toxin". The absence of significant callus growth inhibition with *Pmp* filtrate and only partial inhibition

TABLE 2. The effect of control and culture filtrates (10% v/v) of *Pseudomonas phaseolicola* (*Pp*) on total N, soluble protein, and RNA levels of Manitou bean callus tissue after 14 days[a]

| Treatment | Total N, µg/mg dry wt | Soluble protein, µg/mg dry wt | RNA, µg/mg dry wt |
|---|---|---|---|
| Control filtrate | 33.9 ± 1.9 | 135 ± 5 | 28.3 ± 3.9 |
| *Pp* filtrate | 40.2 ± 2.1 | 107 ± 3 | 25.4 ± 3.6 |

[a] Results are averages from three separate experiments, each composed of eight cultures for each treatment.

with *Ps* filtrate would suggest that general bacterial "staling products" are not responsible. *Pp* filtrates produced from the simple, chemically defined bacterial medium used here inhibited callus growth to about the same degree as filtrates produced from the complex YDC medium used earlier (2). In addition, inhibition of callus tissue growth is induced only by filtrates of toxin-producing strains of *P. phaseolicola* (9).

That callus growth inhibition is due to the "halo toxin" is also evidenced by other similarities between callus tissue and green leaf tissue under the influence of the toxin. Callus tissue grown in *Pp* filtrate had lower levels of soluble protein and higher levels of total N than tissue grown in control filtrate; a similar effect of *Pp* infection in leaf tissue has been reported (7). Particularly significant is the large increase in ornithine, a nonprotein amino acid, in callus tissue grown in *Pp* filtrate. Large increases in ornithine occur in bean tissues exposed to *Pp* infection or to *Pp* filtrates, and this

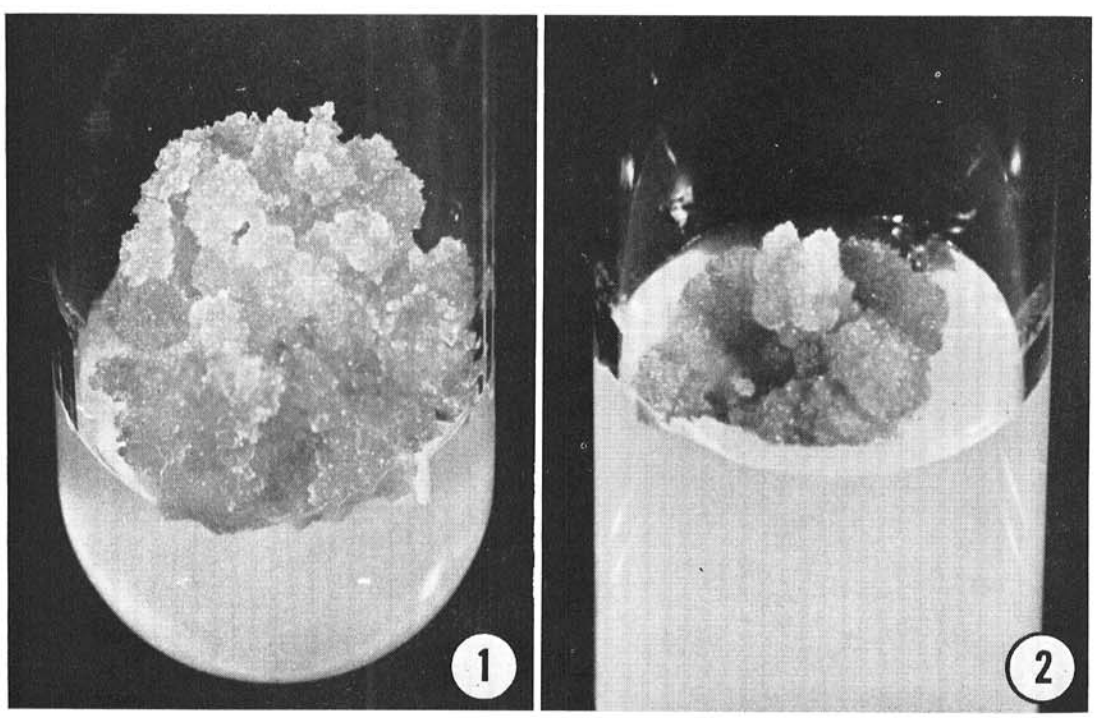


**Fig. 1-2.** **1)** Manitou bean callus tissue grown for 35 days on a medium containing 10% v/v control culture filtrate. **2)** Manitou bean callus tissue grown for 35 days on a medium containing 10% v/v *Pseudomonas phaseolicola* culture filtrate.

TABLE 3. The effect of control and culture filtrates (10% v/v) of *Pseudomonas phaseolicola* (*Pp*) on the levels of ammonia and certain free amino acids in Manitou bean callus tissue grown for 14 days

| Amino compound | Control filtrate | *Pp* filtrate |
|---|---|---|
| | *μmoles/g fresh wt* | |
| Ammonia | 1.7215 | 1.4039 |
| Alanine | 0.1434 | 0.3214 |
| Ethanolamine | 0.1827 | 0.2031 |
| Histidine | 0.0318 | 0.0797 |
| Lysine | 0.0607 | 0.1312 |
| Methionine | 0.0358 | 0.0564 |
| Ornithine | 0.0051 | 0.2814 |
| Serine | 0.1894 | 0.2527 |
| Valine | 0.5274 | 0.6197 |

increase is specific to halo-inducing isolates of halo blight bacteria (6, 8). The increases in alanine, histidine, lysine, methionine, and serine, as well as the decrease in ammonia, which occur in callus tissue grown in *Pp* filtrate, also occur in halo blight-affected leaf tissue (6).

This evidence, together with the fact that stem-callus tissue growth appears to be more sensitive than intact leaves to low toxin concentrations, suggest the feasibility of employing stem-callus to study the mode of action of the bean halo blight toxin.

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