An Ultraviolet-Induced Nontoxigenic Mutant of Pseudomonas phaseolicola of Altered Pathogenicity

Suresh S. Patil, A. C. Hayward, and Rebecca Emmons

Professor, Visiting Professor, and former laboratory assistant Department of Plant Pathology, University of Hawaii, Honolulu, 96822. Present address of the second author: Department of Microbiology, University of Queensland, Brisbane, Australia.

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ABSTRACT

Cultures of a toxigenic isolate of *Pseudomonas phaseolicola,* which is capable of inducing typical halo blight symptoms in bean plants, were irradiated with ultraviolet light. One hundred and eight colonies were randomly selected and tested for their ability to induce halos in needle inoculations of trifoliolate leaves of 'Red Kidney' bean plants. One mutant isolate which in repeated tests failed to induce halos, and the parent isolate were compared with respect to multiplication in vivo, toxin production in culture, nutrition, physiology, and sensitivity to antibiotics. Except for toxin production in culture and the ability to cause systemic chlorosis and systemic invasion in inoculated plants, the mutant is indistinguishable from the wild type. It is proposed that toxigenicity of *P. phaseolicola* is intimately related to its ability to systemically invade susceptible bean plants.

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The extracellular toxin production by *Pseudomonas phaseolicola* induces chlorotic halos in bean leaves which are similar to those caused by the pathogen (5, 13). Although the role of the toxin in chlorosis induction of affected bean tissues is now firmly established, it is still not known whether it has any role in the specificity of *P. phaseolicola* for bean plants. It is a safe assumption that differences in host specificity among phytopathogens reflect variations in their genetic makeup, and that these in turn are extended to differences in their metabolism. The relationship between toxigenicity and pathogenicity of *P. phaseolicola* can be elucidated by doing genetic studies, such as the use of bacteriophages and transductional analysis; however, such studies depend upon the availability of an appropriate, stable, well-defined, mutant which is known to be derived from the original virulent parent isolate.

Here we report the isolation of an ultraviolet-induced mutant of *P. phaseolicola* that fails to produce toxin in culture and even though it multiplies equally as well in vivo as the wild type, it neither causes systemic chlorosis nor invades the inoculated host systemically.

MATERIALS AND METHODS.—*P. phaseolicola,* race 2 strain G-50 (obtained from the late J. Natti) was ultraviolet (UV)-irradiated according to the method of Glover (2) with a Westinghouse Sterilamp (782L-20). One hundred ml of Watanabe's defined medium (18) was inoculated with a loopful of G-50 maintained on yeast-destrose-calcium carbonate (YDC) agar slants. The culture was incubated at 22 C for 16 h on a rotary shaker; the cells were centrifuged (10,000 rpm, 10 min.) and the pellet resuspended in the same salts medium (18) for ultraviolet irradiation. The suspension was adjusted to an optical density (OD) of 0.5 at 670 nm. The viable count obtained from this suspension was 2×10^9 bacteria per ml. From this the following dilutions were made with sterile salts medium: 10^{-1}, 10^{-2}, 10^{-4}, and 10^{-6}. Six ml from each of the dilutions and 6 ml from the undiluted suspension were then transferred to sterile petri plates. The plates were held on the cup of a vortex mixer to gently shake their contents and UV-irradiated for 2 min. Aliquots (0.1 ml) from suspensions were plated on tetrazolium chloride (1 ml/L of 1% TZC) agar plates, and the plates incubated at 22 C. One hundred and eight colonies were chosen at random and streaked on TZC slants. Nutrient broth (Difco) tubes each containing 5 ml were inoculated with the isolates and incubated for 16 h at room temp before centrifuging. The pellet was suspended in sterile deionized water, recentrifuged, and the washed cells resuspended in sterile water. The cell suspensions, which were adjusted to an OD of 0.2 at 670 nm (8), served as inocula.

The isolates were examined for their ability to produce halos by inoculating trifoliolate leaves of 3-wk-old bean plants with each of the isolates in triplicate. Bacterial suspensions were forced into the intercellular spaces with a 25-gauge needle until a water-soaked area of approximately 2 mm in diam was obtained. The plants were incubated for 72 h at 24-26 C in the greenhouse. For the first 24 h after inoculation, plants were covered with plastic bags. At the end of the incubation period, the halos produced by the isolates were scored for size and only those isolates which showed no halos or very small halos were retained. One mutant isolate which in repeated tests consistently failed to cause halos was selected for the studies described here. The wild type is referred to as G50 Tox^+ and the mutant G50 Tox^-.

Mutant and wild type strains of *P. phaseolicola* were examined using the procedures of Lelliott et al. (6) to ascertain whether they belong to their group Ia. Other physiological tests were carried out using the methods of Allen et al. (1). Nutritional screening was done using a modification of the medium of Stanier et al. (16). The medium was essentially the same as that used by Stanier et al. (16), except that the nitrilotriacetic acid was omitted (P. Baumann, *personal communication*). Thirty ml of the medium per petri plate (9-cm diam) was used in screening

tests. Instead of replica plating, approximately 0.1-ml aliquots of dilute distilled water suspensions of the test organisms were applied to the surface of well-dried plates using a Pasteur pipette. The growth of bacteria was recorded after 3, 7, and 14 days incubation at 28 C. Antibiotic sensitivity tests were carried out by applying disks to the surface of plates containing the organism in nutrient agar plus 1% glucose. Zones of inhibition were recorded after 48-h of incubation at 28 C. Sensitivity disks containing the following antibiotics were used: cleocin, mycifradin, lincocin, panmycin, and albamycin (Upjohn Co., Kalamazoo, Mich.); aureomycin sensi-disks (Baltimore Biological Labs., Baltimore, Md.), and erythromycin, streptomycin, bacitracin, polymyxin B, chloromycetin, and penicillin (Difco Labs., Detroit, Mich).

For examining the ability of bacteria to produce toxin, six Fernbach flasks which contained 1.2 liters of autoclaved Watanabe's medium each (10) were inoculated with 8 ml of 48-h starter culture of the desired isolate. The flasks were removed by centrifugation and the supernatant concd to 100-150 ml with a vacuum evaporator. Isolation of the toxin from the concentrate is described elsewhere (10). The final step in this procedure involves gel filtration of the toxin on Sephadex G-10. Final extracts (2 ml) of culture filtrates were applied to a column of Sephadex G-10 (90 X 1.2 cm) and the column eluted with deionized water at a rate of 8 ml/h. Fraction volume was 1.6 ml. The elution volume (V_e) of the toxin is determined by examining the ornithine carbamoyltransferase (OCT) inhibitory activity of the fraction on the forward OCT reaction (17). The physical parameters used in isolation of the toxin from G50 Tox^+ were identical to those used in processing the concentrate from G50 Tox^- cultures. Comparable Sephadex fractions from the G50 Tox^- run were tested against OCT. The two sets of fractions were also tested for halo-inducing activity by putting 10 μl of each of the fractions on trifoliolate leaves of bean plants (9, 11).

The G50 Tox^+ isolates were also compared with respect to symptomatology and multiplication in inoculated bean plants. Bacterial cells for inoculation were obtained by centrifuging 1-day-old cultures that were grown in Watanabe's (18) medium on a shaker (25 C). The pellets were washed, resuspended in sterile deionized water, and the suspensions were adjusted to OD (410 nm) values which corresponded to the OD of standardized suspensions having desired viable cell count.

Two types of inoculation studies were done. In one, undersurfaces of 3-wk-old Red Kidney bean plants were sprayed to runoff with the appropriate cell suspension (10^9 cells/ml), sealed in bags for 24 h and subsequently incubated in the greenhouse at 25-27 C. The second method consisted of carefully spraying under pressure 35.16 – to 42.19 X 10^3 N/m^2 (5-6 psi), the undersurfaces of recently unfolded primary leaves of 5- to 6-day-old bean seedlings with cell suspensions at a close range (15-17 cm). Spraying was continued until a uniform watersoaking of leaves was obtained. In preliminary experiments, we discovered that results were highly reproducible when this technique was employed. This method was used in the quantitative studies of in vivo multiplication of bacteria.

Using the second method, plants were inoculated at two inoculum levels and symptomatology and multiplication of both isolates was studied at each level. The inoculum levels were: (i) wildtype (WT) = 9 X 10^5 cells/ml, and mutant (M) = 8.8 X 10^5 cells/ml; (ii) WT and M = 8 X 10^3 cells/ml. At appropriate intervals five replicate disks (1.5-cm) diam were removed from the inoculated primary leaves with a sterile cork borer, washed either with several volumes of sterile water or given a 2-min soap rinse (Alconox, 0.2%) followed by a 15-s alcohol (70%) rinse, which in turn was followed by a sterile water wash. The latter procedure was used when plants were inoculated with the lower concn of inoculum to eliminate any superficial contaminants. Disks were ground in 20 ml of sterile water in a mortar and pestle. Aliquots (0.1 ml) from appropriate dilutions of these suspensions were put on Tetrazolium (1 ml of 1% TZC/L) agar plates, spread with a sterile glass spreader, and the plates incubated at 27 C for 3 days.

The movement of the two isolates was determined in plants which were inoculated at the highest concn of inoculum used in these studies. The same batch of inoculated plants was used to study the within-plant multiplication and the movement of the bacterial isolates. Three replicate petiolar sections (2-cm-long) from the base of each of the inoculated primary leaves were washed, ground in 10 ml of sterile water, and the dilutions plated as described. Three single leaflets from the first trifiolates of three of the same plants used for taking stem sections were washed in soap, alcohol, and water; ground in a mortar and pestle as above, and their bacterial content determined. Because of the reported difficulty in isolating *P. phaseolicola* from trifoliolates by the grinding technique the infiltration-centrifugation technique of Hildebrand and Schroth (3) was also used when the grinding method failed to yield colonies of the two isolates of *P. phaseolicola* on TZC plates.

RESULTS.—The mutant and wildtype strains of *P. phaseolicola* were indistinguishable in a wide range of physiological, nutritional, and antibiotic sensitivity tests; they were alike in colony form on a variety of agar media. Both isolates were oxidase-negative, produced levan colonies on 5% sucrose nutrient agar, gave a negative reaction in the arginine dihydrolase test, produced a green, diffusible, fluorescent pigment on King's medium B, failed to produce a soft rot in potato slices, and did not contain sudanophilic inclusions. Accordingly, these isolates were assigned to the group Ia of Lelliott et al. (6), and of Misaghi and Grogan (7) which includes *P. phaseolicola*. Other observations made on the two isolates were consistent with the known characters of this species. Neither organism liquefied gelatin, or hydrolysed starch or aesculin. Growth occurred in 2 and 3% NaCl medium, but not in a medium containing 4% NaCl. There was no evidence of lipolytic activity on Tween 80 agar

after 3 days but a weak reaction occurred within 6 days. Deamidation of acetamide did not occur. Tests for production of 3-ketolactose from lactose, nitrite from nitrate, 2-ketogluconate from gluconate, and for liquefaction of a pectate gel at neutral pH were negative. In tests on production of acid from carbohydrates, acid production occurred oxidatively within 1 wk from arabinose, fructose, glucose, galactose, glycerol, mannose, sucrose and melibiose; acid production from rhamnose and raffinose occurred within 2 wk. No acid production occurred within 14 days from cellobiose, dulcitol, erythritol, inositol, lactose, mannitol, maltose, salicin, sorbitol, or trehalose.

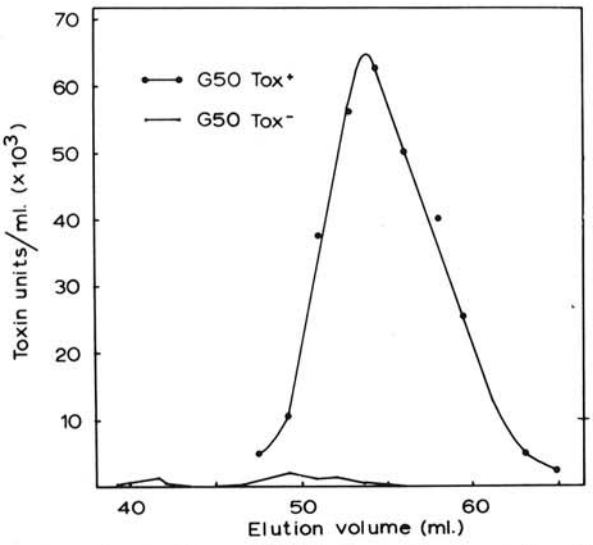

Fig. 1. Ornithine carbamoyl transferase activity of Sephadex G-10 fractions of *Pseudomonas phaseolicola* G50 Tox$^+$ and G50 Tox$^-$ isolates. Two-ml final extracts (10) of the cell-free culture filtrate from the isolates were applied to a column of Sephadex G-10 (90-cm long × 1.2 cm in diam) and eluted with deionized water at a rate of 8 ml/h. Fraction volume was 1.6 ml.

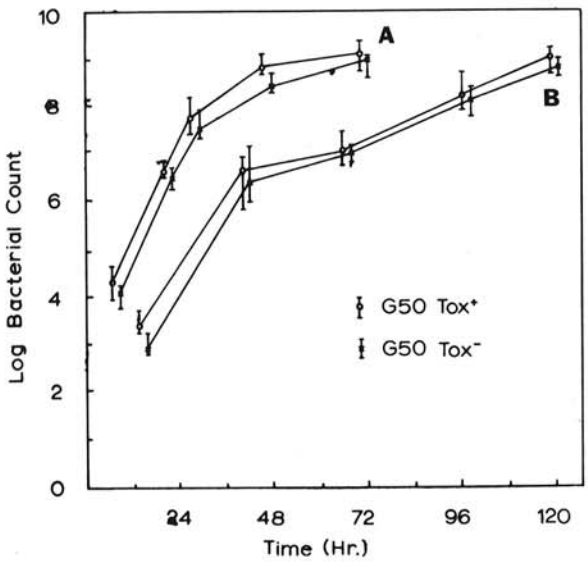

Fig. 2. Multiplication of G50 Tox$^+$ (○), and G50 Tox$^-$ (×) in primary bean leaves. **(A)** inoculum of G50 Tox$^+$, 9×10^5 cells per ml; G50 Tox$^-$, 8.8×10^5 cells per ml. **(B)** inoculum of both isolates, 8×10^3 cells per ml. Five replicate disks (1.2-cm diam) were used to determine bacterial populations. Bars indicate deviation from the plotted mean values.

In nutritional screening tests mutant and wild type isolates utilized the same compounds. Thirty-eight of the 142 organic carbon compounds (27%) served as a sole source of carbon and energy. In some cases heavy growth did not occur until after 7-14 days of incubation; for this reason the results obtained here are more readily comparable with those of Sands et al. (14), and of Misaghi and Grogan (7), who used at least a 2-wk period of incubation, than to those of Stanier et al. (16), who recorded results at 2 and 4 days. The following substrates were utilized as sole carbon source: D-ribose, D-glucose, D-mannose, D-galactose, D-fructose, sucrose, gluconate, saccharate, mucate, glucuronate, galacturonate, propionate, heptanoate, caprylate, pelargonate, caprate, malonate, succinate, fumarate, DL-malate, DL-β-hydroxybutyrate, citrate, α-ketoglutarate, pyruvate, aconitate, glycerol, *p*-hydroxybenzoate, quinate, L-α-alanine, D-α-alanine, L-serine, L-aspartate, L-glutamate, L-arginine, γ-amino butyrate, L-proline, putrescine, and trigonelline.

Substrates which did not serve as sole carbon source were as follows: D-xylose, D-arabinose, α-D(+)fucose, L-rhamnose, trehalose, maltose, cellobiose, melibiose, lactose, inulin, salicin, N-acetyl glucosamine, formate, acetate, butyrate, valerate, isovalerate, caproate, oxalate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, D(−)tartrate, L(+)tartrate, *meso*-tartrate, DL-lactate, DL-glycerate, laevulinate, citraconate, itaconate, mesaconate, erythritol, mannitol, sorbitol, *meso*-inositol, adonitol, propylene glycol, 2,3-butylene glycol, methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, geraniol, D-mandelate, L-mandelate, benzoyl formate, benzoate, *o*-hydroxy benzoate, phenyl ethanediol, phenol, glycine, β-alanine, L-threonine, L-leucine, L-isoleucine, L-norleucine, L-valine, L-lysine, L-ornithine, L-citrulline, DL-α-amino butyrate, DL-α-amino valerate, δ-amino valerate, L-histidine, L-tyrosine, L-phenyl alanine, L-tryptophan, DL-kynurenine, kynurenate, anthranilate, *m*-amino benzoate, *p*-amino benzoate, methylamine, ethanolamine, benzylamine, spermine, histamine, butylamine, α-amylamine, 2-amylamine, pentylamine, betaine, sarcosine, creatine, hippurate, pantothenate, acetamide, nicotinate, nicotinamide, allantoin, adenine, thymine, uracil, alginate, chitin, and cellulose.

In antibiotic sensitivity tests both isolates were resistant to cleocin, 2 μg/disk; lincocin, 2 μg/disk; erythromycin, 2 μg/disk; bacitracin at 2 to 10 units/disk; polymyxin B, 50 units/disk; and penicillin,

10 units/disk. Both were sensitive to the following antibiotics and gave zones of comparable size in each case: mycifradin, 30 μg/disk, panmycin, 30 μg/disk; albamycin, 30 μg/disk; aureomycin, 30 μg/disk erythromycin, 15 μg/disk; streptomycin at 2 and 10 μg/disk; polymyxin B, 300 units/disk; and chloromycetin at 5 and 30 μg/disk.

Results of the experiment to determine the ability of the wild type and mutant isolates to produce toxin in culture are shown in Fig. 1. The data show that in the peak column fraction of G50 Tox$^+$ there were over 65,000 units/ml of toxin; whereas, no detectable toxin was present in the comparable fraction of G50 Tox$^-$. Also, only G50 Tox$^+$ fractions showed halo-inducing activity.

When 3-wk-old bean plants were inoculated by spraying till runoff (without forcing the bacteria in the intercellular spaces) both isolates caused water-soaking in inoculated leaves. Generally, younger trifoliolate leaves had greater numbers of lesions than older ones. But even in leaves of roughly the same age, there was considerable variation in the number of infection loci per cm^2 of leaf area. Therefore, no attempt was made to quantitate the results of these experiments. Even though both isolates induced water-soaking in inoculated plants, only G50 Tox$^+$ (but not G50 Tox–) induced systemic chlorosis.

The results of the multiplication studies in which the undersides of primary leaves were sprayed under pressure to force the inoculum in intercellular spaces are shown in Fig. 2. At the high inoculum concn (Fig. 2a) (9×10^5 cells/ml for G50 Tox$^+$ and 8.8×10^5 cells/ml for G50 Tox$^-$), both isolates multiplied logarithmically up to 36 h, after which time the rate of multiplication for both isolates slowed by approximately the same degree. The first trifoliolates of plants inoculated with the wild type showed systemic chlorosis and severe stunting, but plants inoculated with the mutant showed no systemic chlorosis and no stunting.

At the lower inoculum concn (8×10^3 cells/ml) again, both isolates multiplied at the same rate although the slopes of these curves were slightly less steep (Fig. 2b) than those shown in Fig. 2a. The rate of growth for both isolates is logarithmic for about 40 h after which there is a change in slopes of the curves. Maximum cell numbers (7×10^8 cells) which in the case of high inoculum were attained by about 48 h, were not attained at the low inoculum concn until 96 h after inoculation. At the low inoculum level even the wild type failed to induce either systemic chlorosis or stunting the trifoliolate leaves were green and of the same size as that of the mutant-inoculated plants.

Results of determinations of bacterial populations in petiole sections and in single leaflets of trifoliolate leaves are shown in Table 1. Seven days after inoculation at the high inoculum level, there were approximately 7×10^7 bacteria per petiole section for the wild type and mutant respectively. Nine days after inoculation the wild type population per petiole section appeared to decrease slightly whereas the mutant population decreased somewhat more. From leaflets of trifoliolate leaves, 3×10^7 cells/leaflet of G50 Tox$^+$ were recovered 7 days after inoculation. No bacteria could be isolated from leaflets of trifoliolates of plants inoculated with the mutant. Even when leaflets from mutant-inoculated plants were subjected to the infiltration centrifugation technique of Hildebrand and Schroth (3), no mutant bacteria were recovered.

TABLE 1. Bacterial populations in petiolar sections and in leaflets of first trifoliolate leaves of bean plants inoculated with G50 Tox$^+$ and G50 Tox$^-$

Bacterial isolate	Bacterial count			
	Petiole section[a]		Leaflet[b]	
	7 days[c]	9 days	7 days	9 days
G50 Tox$^+$	7×10^7	6×10^7	3×10^7	3.5×10^7
G50 Tox$^-$	8.6×10^7	1.6×10^7	0	0

[a]Mean bacterial count of three replicate 2-cm-long petiole sections taken from the bases of petioles of three primary leaves 7 and 9 days after inoculation.

[b]Mean bacterial count of three replicate single leaflets taken from the first trifoliolate leaf 7 and 9 days after inoculation of primary leaves.

[c]Plants used in this experiment were from the same batch of inoculated plants from which data in Fig. 2A were obtained.

DISCUSSION.–The mutant and wild type strains of *P. phaseolicola* conform to the characteristics of this species given by Lelliott et al. (6); Misaghi and Grogan (7); and Sands et al. (14). *P. phaseolicola* is among the least nutritionally versatile of the plant pathogenic pseudomonads. Sands et al. (14) found that eight strains of this species utilised 22-31% of the 165 carbon sources tested in their study, which compares well with the 27% of a comparable range of carbon sources obtained in this study. A systematic comparison of the mutant and wild type strains of *P. phaseolicola* showed no differences in cultural or nutritional properties which correlated with the altered pathogenicity or toxigenicity in the mutant strain.

To our knowledge, this is the first report of UV-induced, stable, nontoxigenic mutant derived from a toxigenic isolate of *P. phaseolicola*. Even though the mutant is nontoxigenic and lacks the ability to induce systemic chlorosis, it multiplies in inoculated primary bean leaves at approximately the same rate, and attains roughly the same maximum population level as the parent isolate at both the high and the low inoculum concns (Fig. 2A, B). Omer and Wood (8) showed that both race 1 and race 2 of *P. phaseolicola* multiply at the same rate for 4 days in inoculated primary leaves of 'Red Mexican' bean plants. However, race 1 to which Red Mexican is resistant, stops multiplying after 4 days and does not attain the same maximal population (10^9 cells) as the race 2 bacteria. Since the semilog plots (Fig. 2) for

growth of G50 Tox$^+$ and G50 Tox$^-$ are almost identical, both isolates must be considered equally compatible with Red Kidney bean. When this is viewed in light of the nutritional data on the two isolates, it becomes clear that the mutant we have isolated is not a spurious contaminant but an organism which is closely related to the parent isolate.

The mutation clearly separates the pathogenicity of *P. phaseolicola* into two distinct traits: One, the ability to multiply in vivo; and two, the ability to cause systemic chlorosis and to invade the host systemically. The mutant has retained the former trait, but has lost the latter and a loss of the only other observable characteristics to which this can be correlated is toxigenicity. Therefore, it is tempting to suggest that the loss of systemicity is the result of the loss of toxigenicity by the mutant isolate.

Although there is no direct evidence to support our contention that there is a cause-effect relationship between toxigenicity and systemicity, there is considerable circumstantial evidence which supports that contention. Hoitink et al. (5) hinted at a positive correlation between virulence of several isolates of *P. phaseolicola* and their ability to produce toxin in vivo and in culture. Rudolph (12) reported that prior introduction of toxin into leaves of plants which were resistant to *P. phaseolicola* allowed the pathogen to multiply in such leaves equally as well as in untreated susceptible leaves. Although this observation does not explain the role of the toxin in systemicity, it does indicate that the toxin is involved in more than the induction of chlorosis in affected plants.

There is other indirect supporting evidence. At least one report (4) shows that even in susceptible bean cultivars, systemicity does not occur unless an optimum initial concn of bacteria is used to inoculate the plants. Hill et al. (4) reported that when the inoculum concn of a race 2 isolate of *P. phaseolicola* was low (5×10^6 cells per ml) no systemic chlorosis was observed in a susceptible (Pinto UI 111) bean cultivar. However, at a higher inoculum concn (2×10^8 cells per ml) of the pathogen, plants of the same cultivar showed systemic chlorosis. This indicates that there is a minimum threshold of pathogenic population, and (presumably) also that of the toxin concn before systemicity can occur. However, this assumes that the toxin was produced in vivo and its concn was proportional to pathogenic growth in the experiments of Hill et al. (4). We have already shown that in vivo the race 2 bacterium (G50 Tox$^+$) is capable of producing a toxin which is identical in its physico-chemical properties to the toxin produced in culture (Patil, *unpublished*). Therefore it would be reasonable to assume that in the experiments of Hill et al. (4) toxin concn was higher in plants inoculated at the high inoculum level than at the low inoculum level.

The results of the current multiplication experiments further illustrate the point. Our results are not strictly comparable to those of Hill et al. (4) because we used a method of inoculation (leaf water-soaking by infiltration under pressure) which differed from that (spraying till runoff) used by the above authors. This may explain why we observed systemic chlorosis at a much lower concentration compared to that used by Hill et al. (4). At the high concn (9×10^5 cells per ml) of G50 Tox$^+$ both systemic invasion and systemic chlorosis occur. Further, data in Fig. 2 show that the maximal population level in case of the high inoculum concn is attained faster than at the low concn. Obviously, it is not the growth rate (slopes of growth curves for both isolates in Fig. 2A as well as in Fig. 2B are about the same) but rather the attainment of a certain optimum population level within a certain time period that appears to induce systemicity.

The question which now arises is, how unique is the mutant? In their extensive work Schroth et al. (15) reported several naturally occurring isolates of *P. phaseolicola* which cause water-soaking in certain bean cultivars, but do not induce systemic chlorosis. At first glance, these isolates might appear to be naturally occurring nontoxigenic isolates of *P. phaseolicola*. However, the current results caution against drawing such a conclusion, because even a known toxin-producing isolate (G50 Tox$^+$) causes only water-soaking at low inoculum levels. Therefore, the naturally occurring isolates and the mutant must be compared with respect to in vivo quantitative multiplication as well as toxin production before any conclusions regarding their similarity can be drawn.

What is more important to this discussion is the observation of Schroth et al. (15) that "in general, systemic symptoms were noted more commonly with host-pathogen combinations where there was severe infection of leaves". Our finding that G50 Tox$^+$ causes systemicity only at the high inoculum concentration (severe infection) is consistent with their observation.

Schroth et al. (15) did, however, report exceptions to their general observation. Certain isolates caused water-soaking and systemic chlorosis in some cultivars in the absence of severe infection. On the other hand, these same isolates caused no systemic chlorosis in other cultivars in spite of severe infection of inoculated leaves. These results point to the possibility that host tissues of different cultivars may control pathogenic growth and toxin production differentially. Thus, to obtain the true measure of the involvement of the toxin of *P. phaseolicola* in the systemic invasion of bean plants by that bacterium, both in vivo growth and toxin production will have to be studied. Studies along these lines are currently in progress in this laboratory.

LITERATURE CITED

1. ALLEN, R. N., A. C. HAYWARD, W. J. HALLIDAY, and J. FULCHER. 1970. Bacterial blight of Vicia sativa: aetiology of the disease and identification of the pathogen. Aust. J. Biol. Sci. 23:597-606.
2. GLOVER, S. W. 1968. Mutation. Pages 13-27. *in* R. C. Clowes and W. Hays, eds. Experiments in microbial genetics. Blackwell Scientific Publications, New York.
3. HILDEBRAND, D. C., and M. N. SCHROTH. 1971. Isolation of Pseudomonas phaseolicola from bean

leaves exhibiting systemic symptoms. Phytopathology 61:580-581.

4. HILL, K., D. P. COYNE, and M. L. SCHUSTER. 1972. Leaf, pod, and systemic chlorosis reactions in Phaseolus vulgaris to halo blight controlled by different genes. J. Am. Soc. Hortic. Sci. 97:494-498.

5. HOITINK, H. A. J., R. L. PELLETIER, and J. G. COULSON. 1966. Toxemia of halo blight of beans. Phytopathology 56:1062-1065.

6. LELLIOTT, R. A., E. BILLING, and A. C. HAYWARD. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bact. 29:470-489.

7. MISAGHI, I., and R. G. GROGAN. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.

8. OMER, M. E. H., and R. K. S. WOOD. 1969. Growth of Pseudomonas phaseolicola in susceptible and resistant bean plants. Ann. Appl. Biol. 63:103-116.

9. PATIL, S. S., P. E. KOLATTUKUDY, and A. E. DIMOND. 1970. Inhibition of ornithine carbamyl transferase from bean plants by the toxin of Pseudomonas phaseolicola. Plant Physiol. 46:752-753.

10. PATIL, S. S., L. Q. TAM, and P. E. KOLATTUKUDY. 1971. Isolation and mode of action of Pseudomonas phaseolicola toxin, Pages 365-372. *in* R. K. S. Wood, A. Ballio, and A. Graniti, eds. Phytotoxins in plant diseases. Academic Press, New York. 530 p.

11. PATIL, S. S., L. Q. Tam, and W. S. SAKAI. 1972. Mode of action of the toxin from Pseudomonas phaseolicola. I. Toxin specificity, chlorosis, and ornithine accumulation. Plant Physiol. 49:803-807.

12. RUDOLPH, K., 1971. The halo blight toxin of Pseudomonas phaseolicola: Influence on host-parasite relationship and counter effect of metabolites. Pages 373-375. *in* R. K. S. Wood, A. Ballio, and A. Graniti, eds. Phytotoxins in plant disease. Academic Press, New York. 530 p.

13. RUDOLPH, K., and M. A. STAHMANN. 1966. The accumulation of L-ornithine in halo-blight infected bean plants (Phaseolus vulgaris L.), induced by the toxin of the pathogen Pseudomonas phaseolicola (Burkh.). Phytopathol. Z. 57:29-46.

14. SANDS, D. C., M. N. SCHROTH, and D. C. HILDEBRAND. 1970. Taxonomy of phytopathogenic pseudomonads. J. Bact. 101:9-23.

15. SCHROTH, M. N., V. B. VITANZA, and D. C. HILDEBRAND. 1971. Pathogenic and nutritional variation in the halo blight group of fluorescent pseudomonads of bean. Phytopathology 61:852-857.

16. STANIER, R. Y., N. J. PALLERONI, and M. DOUDOROFF. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43:159-271.

17. TAM, L. Q., and S. S. PATIL. 1972. Mode of action of the toxin from Pseudomonas phaseolicola. II. Mechanism of inhibition of bean ornithine carbamoyltransferase. Plant Physiol. 49:808-812.

18. WATANABE, M. 1967. Studies on the nutritional physiology of Xanthomonas oryzae. Ann. Phytopathol. Soc. Jap. 28:201-206.