A Seedling Bioassay Chamber for Determining Bacterial Colonization and Antagonism on Plant Roots

P. S. Randhawa and N. W. Schaad

Postdoctoral research associate and professor, respectively, Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow 83843. Present address of first author: USDA, ARS, Horticultural Science Institute, Fruit Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705.

This investigation was a project under NC-135 and was supported in part by USDA Special Grant PL-89-106 on Biological Control of Pests.

Published as Idaho Agricultural Experiment Station Journal Series Article 84712.

Accepted for publication 4 October 1984 (submitted for electronic processing).

ABSTRACT

Randhawa, P. S., and Schaad, N. W. 1985. A seedling bioassay chamber for determining bacterial colonization and antagonism on plant roots. Phytopathology 75:254-259.

A seedling bioassay is described for identifying bacteria or other microorganisms antagonistic to plant pathogens based upon competitive colonization of roots. A seedling bioassay (SBA) chamber is constructed by dividing a square petri plate into two compartments with a sterile glass rod held in place with a small amount of sterile molten water agar. Agar medium is added to one compartment and a perforated, absorbant paper pouch, folded to hold seeds and soil, is attached to a glass platform in the other compartment. The chamber is incubated vertically; the glass rod divider is horizontal, the solidified agar medium is in the compartment beneath it, and the paper pouch is in the compartment above it. Seeds infested with possible antagonists are germinated, the emerging radicles are inoculated with bacterial or fungal pathogens, and the germinating seeds are placed in the pouch in the upper compartment of the SBA chamber. After 48–72 hr, roots emerge through the pouch, cross the glass rod partition, and begin growing down the surface of the agar. An appropriate selective agar medium can be used to detect root colonization and pathogen antagonism along the root. Roots can also be assayed for bacterial populations by excision, comminution in water, and dilution plating on agar media. For fungal pathogens, antagonism is expressed by prevention of symptoms in the seedlings. Of 122 bacteria obtained from washings of various seeds, 82 colonized collard roots and 20 of the colonizers prevented growth of *Xanthomonas campestris* pv. *campestris*. Similar results were obtained with *Pseudomonas solanacearum* on tomato roots. Relatively few bacteria that produced inhibition zones in agar colonized roots of seedlings. All bacterial strains identified as antagonists in SBA chamber were root colonizers, but all colonizers were not antagonists. The SBA chamber provides a simple, inexpensive method of screening bacteria for their ability to colonize roots and antagonize specific pathogens.

Additional key words: biological control, bacteria, antagonists.

Biological control of soilborne plant pathogens by utilizing microbial antagonism has created considerable interest in its potential value, but it has had only limited application (1,2,9,13,16,22,25). One reason for this limited use is that techniques for identifying microorganisms antagonistic to plant pathogens are based on inhibition of the pathogen on artificial substrate rather than on the host (2,5,7). There is little correlation between antagonism on an agar medium and active plant protection (6,8,24). Gemrich and Vandestreek (6) observed that *Bacillus uniflagellatus*, which produced an antibiotic that was toxic to many pathogens in vitro, was ineffective in controlling diseases of beans, peas, and cotton. On the other hand, a bacterial strain that protected potato plants against *Pseudomonas solanacearum* was incapable of inhibiting that pathogen on agar (8). One explanation for the failure of many bacteria to protect plants against infection is their inability to survive and migrate along the surfaces of growing roots (23). Antibiotic-producing rhizosphere strains are apparently highly effective in reducing harmful flora on potato (12) and wheat (25) roots, thereby increasing yields (4). *Erwinia carotovora* is displaced from potato roots by plant-growth-promoting rhizobacteria (11). Since only certain bacteria can migrate along the roots of plants (15), selection of antagonists should be from among those able to colonize roots.

This work was initiated, therefore, to develop a rapid and reliable bioassay for selecting antagonists based on their ability to colonize roots and antagonize plant pathogens thereon. A preliminary report has been published (18).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Seedling bioassay (SBA) chamber. A 100 × 100 × 15-mm square plastic petri dish (internal dimensions 89 × 89 × 15 mm) is divided into two compartments, one 45 × 89 mm and the other 38 × 89 mm with a 6-mm-diameter sterile glass rod (Fig. 1). The glass rod is fixed in place with sterile molten water agar, and the smaller compartment is filled with 12 ml of agar medium. For holding test materials (seed or soil), an absorbent paper pouch is constructed using 75 × 36-mm and 75 × 18-mm strips of tan regular-weight and blue heavy-weight (respectively) seed germination papers (Anchor Paper, 480 Broadway, St. Paul, MN 55101), and an 87 × 24-mm strip of extra-heavy aluminum foil (A in Fig. 1 and *a* in Fig. 2). The regular-weight paper (*a* in Fig. 2) is folded lengthwise to form a 12-mm pouch and the remaining 12-mm of paper is hung over the blue paper (*b* in Fig. 2). The pouch and the blue supporting paper are held together by folding the 87 × 24-mm strip of aluminum foil over the upper open end and each side (A in Fig. 1 and *c* and *d* in Fig. 2). Several 1- to 2-mm-diameter holes are made in the bottom of the pouch with a dissecting needle. The pouch is then attached to a glass support chamber (B in Fig. 1 and e in Fig. 2) with double-stick tape. To construct glass support chambers, 3-mm-diameter glass rods are attached with silicone glue to three edges of the upper surface of a 25 × 75-mm glass slide. Another slide is glued onto the glass rods to form a 22 × 75 × 3-mm chamber open along one 75-mm axis (B and *c* in Fig. 1 and *e* in Fig. 2). The entire unit (support chamber with attached pouch) (C in Fig. 1 and *f* in Fig. 2) is autoclaved and attached to the petri plate adjacent and parallel to the glass rod (D in Fig. 1), with two drops of silicone glue, so that the blue germination paper touches the glass rod (Fig. 3). This leaves a 6-mm space between the pouch and glass rod. If the blue paper is not touching, it can be pulled to the glass rod with sterile forceps.

Origin of test strains and preparation of inocula. Bacteria to be tested for root colonization and antagonism were obtained from commercial seed lots and from apple blossoms collected in an orchard in Parma, ID. One to 2 g of seed or blossoms were shaken for 1–3 min at room temperature (22 ±1 C) in 5 ml of saline containing 0.05% Tween-20 . The washings were decanted, serially diluted, and spread with an ethanol-flamed glass rod onto triplicate plates of Difco nutrient agar plus 0.5% glucose (NGA) by using a turntable (21). After 3 days at 30 C, types of bacterial colonies most abundant (Table 1) on NGA plates with 50–200 colonies were removed, purified, and stored on slants of NGA at 2–5 C.

Inocula for bacteria were prepared by growing cultures on NGA plates for 24 hr at 30 C. The resulting cells were transferred with a sterile toothpick into a drop of sterile water in a plastic petri dish lid to obtain a turbid cell suspension. For *X. campestris* B-24 and *P. solanacearum* K-60 STR^r, cells were added to sterile distilled water, agitated with a Vortex mixer, and adjusted to 0.05 OD at 535 nm (Baush and Lomb colorimeter). Strain K-60 STR^r, which is

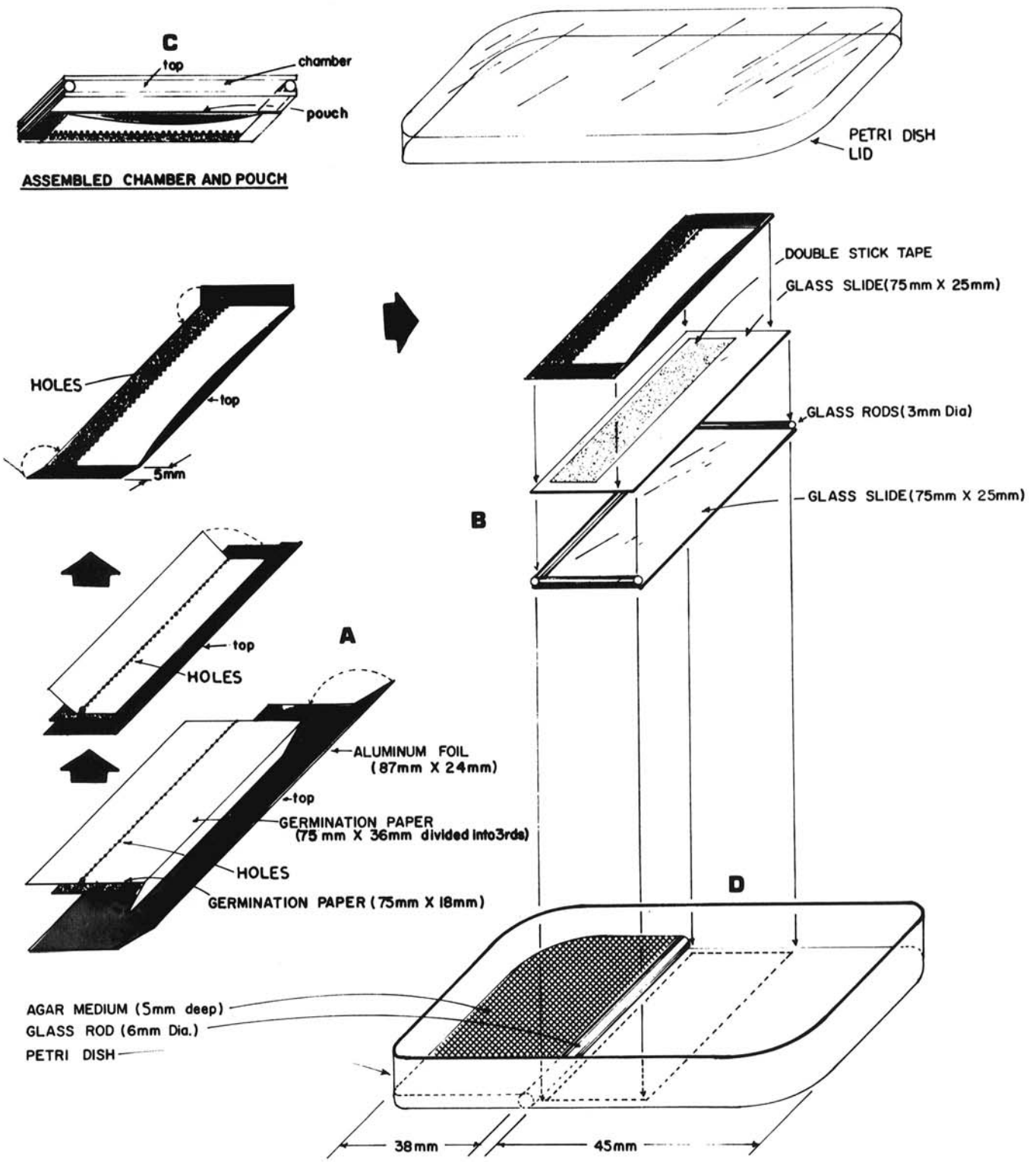
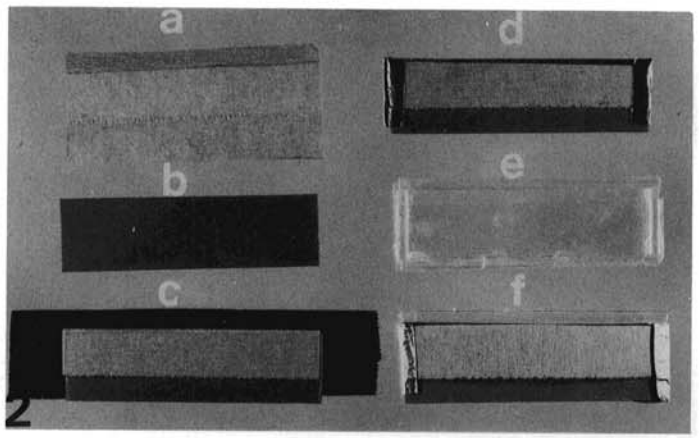


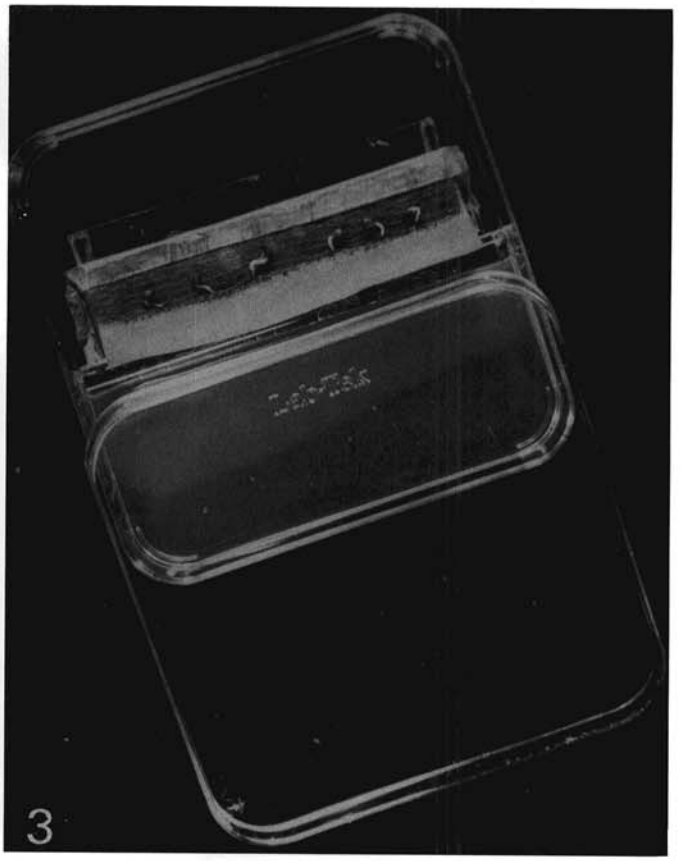
Fig. 1. Diagram of components of a seedling bioassay chamber. An absorbent paper pouch (A) made of seed germination paper and held together with aluminum foil is attached to a glass support chamber (B). The assembled chamber and pouch (C) is autoclaved and attached with silicone glue to a square plastic petri dish (already containing agar medium in the smaller compartment) above and adjacent to the glass rod (D).

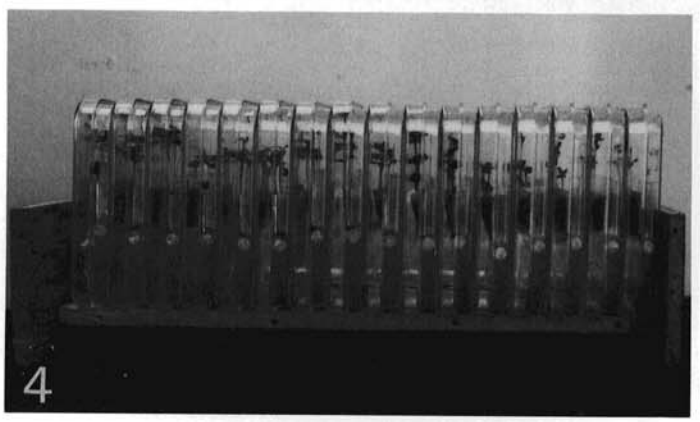
resistant to streptomycin at 100 mg/L, was derived from strain K-60 (A. Kelman, University of Wisconsin, Madison). Inoculum of *Rhizoctonia solani* Kühn strain 6212 AG-4 (D. Sumner, Georgia Agricultural Experiment Station, Tifton) was prepared by growing the fungus in 3% cornmeal sand (CMS = yellow corn meal 3 g, washed sand 100 g, deionized water 15 ml) in 250-ml flasks for 7 days at 22 ±2 C. The flasks were shaken by hand daily. The inoculum was stored at 2–5 C for up to 1 mo before use.

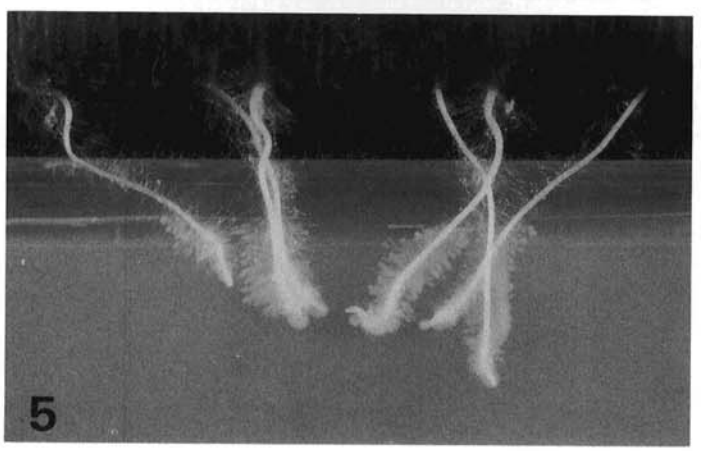
Growth of seedlings and application of inoculum. All seeds were surface-disinfested for 10 min in a 1:10 dilution of commercial Clorox (5.25% NaOCl), washed in sterile water, dried in a laminar-flow hood, and assayed by plating on NGA to verify that no bacteria were present. These seeds, presumably free from bacteria, were stored at 2–5 C in a sterile petri dish until used. Ten to 15 seeds were treated with each test strain by dipping them individually in a drop of inoculum suspension for 1 min at room temperature. The inoculated seeds were germinated on sterile wet blotter papers in a petri plate in the dark at 30 C for 24–60 hr as needed to produce a visible radicle. Each pouch was opened aseptically by unfolding the aluminum foil in a laminar flow hood and watered with 1.5 ml of sterile water. Six seeds with radicles 2- to 4-mm long were carefully placed into the pouch with the radicles positioned toward a hole (Fig. 3). The SBA chamber was kept in a horizontal position for easy operation (Fig. 4).

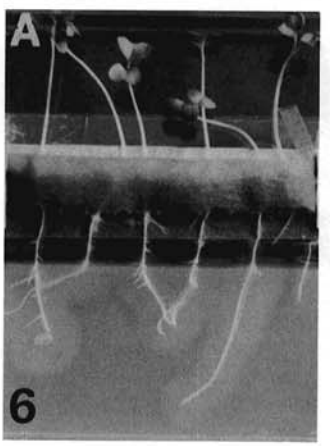
For inoculation with bacterial pathogens, the radicles of the germinating seeds were dipped for 1–2 sec in a freshly prepared

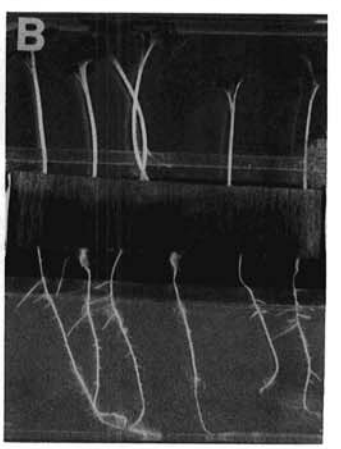












Figs. 2–6. Construction and utilization of the seedling bioassay chamber. **2,** Components and construction of the paper pouch. a) Regular-weight, tan seed germination paper. b) Heavy-weight, blue germination paper. c) Regular-weight paper folded to form a pouch and placed over the heavy-weight paper is placed on a strip of heavy aluminum foil. d) Aluminum foil folded over pouch and heavy-weight paper. e) Glass support chamber with a strip of double-stick tape. (f) Assembled pouch attached (with the tape) to the support chamber. **3,** Bioassay chamber with pouch opened to show germinating seeds and holes in pouch for easy exit of elongating seed radicals. **4,** Seedling bioassay chambers held in a vertical position. **5,** Roots of collards cultivar Georgia emerging from pouch of seedling bioassay chamber and growth of a root-colonizing bacterium on nutrient agar medium. **6, A,** Inoculated control showing strain B-24 growing profusely around roots (note zones of starch hydrolysis). **B,** Antagonism of *Xanthomonas campestris* pv. *campestris* strain B-24 on collard roots by strain RC-125, a root-colonizing bacterium, completely inhibiting growth of B-24 in the seedling bioassay chamber.

suspension of inoculum and placed in a pouch as described above. For *R. solani*, germinated cucumber seeds were positioned in the pouch and 0.7 to 1.0 g of CMS inoculum was poured over the seeds. The pouches were closed, and the SBA chambers were held upright in a rack and incubated at room temperature (22 ±2 C) with a 12-hr photoperiod. After 60 hr, 0.7 ml of sterile water was added dropwise to the blue germination paper in a laminar-flow hood. Alternatively, 1.0 ml of water was added to the glass support chamber and a 50 × 3-mm wick (made of regular-weight germination paper) was attached for slow transfer of water to the pouch. After incubation for 4–8 days, bacterial growth along the excised or intact roots was noted and disease symptoms on seedlings were recorded.

Colonization and antagonism. To study colonization and antagonism on roots, the lower part of the SBA chamber was filled with different agar media depending upon the pathogen being tested. For *X. campestris*, nutrient starch agar (21) or a semiselective basal starch antibiotic agar (BSAA) (20) without cycloheximide was used. Seeds of collard (*Brassica oleracea* var *acephala* DC. 'Georgia L.S.') were treated with test strains and germinated for 24 hr. Six uniformly germinated seeds inoculated with B-24 or uninoculated, respectively, were transferred to pouches as described. To determine numbers of colony-forming units (cfu) of B-24 added to each seed, five additional seeds were inoculated with B-24 only. Each of the five seeds was suspended in 1.0 ml of saline, and the suspension was assayed for B-24 by dilution plating onto triplicate plates of NGA.

After incubation in the SBA chambers for 5–6 days, growth of test bacteria and B-24 was observed (Fig. 5). In addition, growth of B-24 was evidenced by zones of starch hydrolysis (Fig. 6A). Test strains that resulted in reduced visible growth of B-24 in comparison to profuse growth of B-24 in the controls were rated as antagonistic. In a comparative evaluation for inhibition on agar, strains were spotted onto plates of NGA with sterile toothpicks. After 24 hr at 30 C, an inoculum suspension of strain B-24 (approximately 10^8 cfu/ml) was oversprayed on the agar surface with a sterile chromatographic sprayer in a fume hood. The plates were incubated at 30 C for 36 hr, and zones of inhibition around test colonies were measured.

For *P. solanacearum*, seeds of hybrid tomato (*Lycopersicon esculentum* Mill 'Tempo') were used. SBA chambers were prepared with water agar in the lower compartment. A strip of regular-weight seed germination paper was laid over the water agar. The seeds treated with test bacterial strains were germinated for 60 hr. Germinated seeds with or without inoculation with K-60 STR^r for antagonism or colonization, respectively, were transferred to the pouches. After 4 days at 30 C, roots were sampled. To determine bacterial colonization, the roots beyond the glass rod were excised and placed on NGA at 30 C for 36 hr to visualize bacterial growth. To evaluate antagonism, distal 10-mm portions of three roots that had grown at least 45 mm were cut with a sterile scalpel. These root portions were transferred to 1 ml of saline and vigorously shaken for 30–40 sec. The suspension was serially diluted to 10^{-3}, and 0.1 ml from each dilution was plated onto NGA plates containing 100 μg of streptomycin per milliliter. After 3–4 days of incubation, the colony-forming units of K-60 STR^r were counted and percent reduction was calculated in relation to inoculated control (K-60 STR^r only). Strains that reduced K-60 STR^r colonies by 95% or more were counted as inhibitory.

For *R. solani*, seeds of cucumber (*Cucumis sativus* 'Chicago Pickling') were treated with bacteria, germinated for 24 hr, and transferred to pouches. CMS inoculum of *R. solani* diluted 1:5 with sterile sand, sufficient to cover the seeds (0.7 to 1.0 g), was added to the pouch with a sterile spatula. The pouches were carefully closed to avoid sand spills outside the pouch. After incubation for 4–6 days, disease symptoms were recorded. Any SBA chambers with test strains that prevented seedling collapse were further incubated for up to 8 days to study extended protection. In addition, roots of seedlings with delayed or no symptoms were excised and placed on fresh potato-dextrose agar (PDA) and PDA containing 200 μg of streptomycin per milliliter to observe any growth of bacteria and *R. solani* along the roots. Our experiments on colonization and antagonism were repeated at least once.

RESULTS

Growth of seedlings. Choosing only seeds with emerging radicles resulted in uniform development of seedlings with roots emerging through the pouch within 24 hr and reaching the lower compartment of the SBA chamber within 48–60 hr. Further growth varied with the agar medium and species of plant. Growth of collard roots was relatively slow on nutrient starch agar compared to growth on water agar and BSAA. Tomato roots grew well on water agar but not on NA or tetrazolium agar (10). Cucumber roots grew well on water agar. Roots of all test plants reached the lower end of the SBA chamber in 80–96 hr when water agar was present. Secondary roots usually became visible after 4 days. All seedlings grew upright, appeared vigorous, had green cotyledons, and did not require added nutrients for normal growth during the 4–8 day test period.

Application of inocula. Collard seeds inoculated only with *X. campestris* had a mean of 8×10^3 cfu per seed. Adding 5 μl of a suspension of *X. campestris* to each seed prior to sowing in the pouch or to the root after its emergence from the pouch (ie, behind the root tip), gave inconsistent results. Some roots below the glass rod contained the pathogen and others did not. On the other hand, dipping radicles of germinated seeds of collard and tomato in suspensions of *X. campestris* and *P. solanacearum*, respectively, resulted in consistent movement of the pathogens along developing roots. Adding inoculum of *R. solani* diluted 1:5 with sterile sand resulted in consistent collapse of cucumber plants after 3–4 days,

TABLE 1. Colonization of collard roots and inhibition of *Xanthomonas campestris* pv. *campestris* by bacteria isolated from various plant seeds

Seed source of bacteria	Strains tested (no.)	Strains (no.) positive for:	
		Colonization[a]	Inhibition[b]
Crucifer	80	51	10
Tomato	31	23	7
Pepper	3	1	0
Wheat	8	7	3
Total	122	82	20

[a] Bacteria-free collard seeds were amended with test strains, germinated for 24 hr at 30 C and placed in pouches in seedling bioassay chambers. Observations were made for presence of test strains along the growing root on nutrient starch agar.

[b] Seeds coated with test strains were germinated for 24 hr at 30 C, inoculated with *X. campestris* pv. *campestris* strain B-24 and grown in seedling bioassay chambers. Strains that reduced growth of B-24 along roots, in comparison to growth in control, were counted as inhibitory.

TABLE 2. Colonization of tomato roots and inhibition of *Pseudomonas solanacearum* K-60 STR^r by strains of bacteria isolated from various plant sources

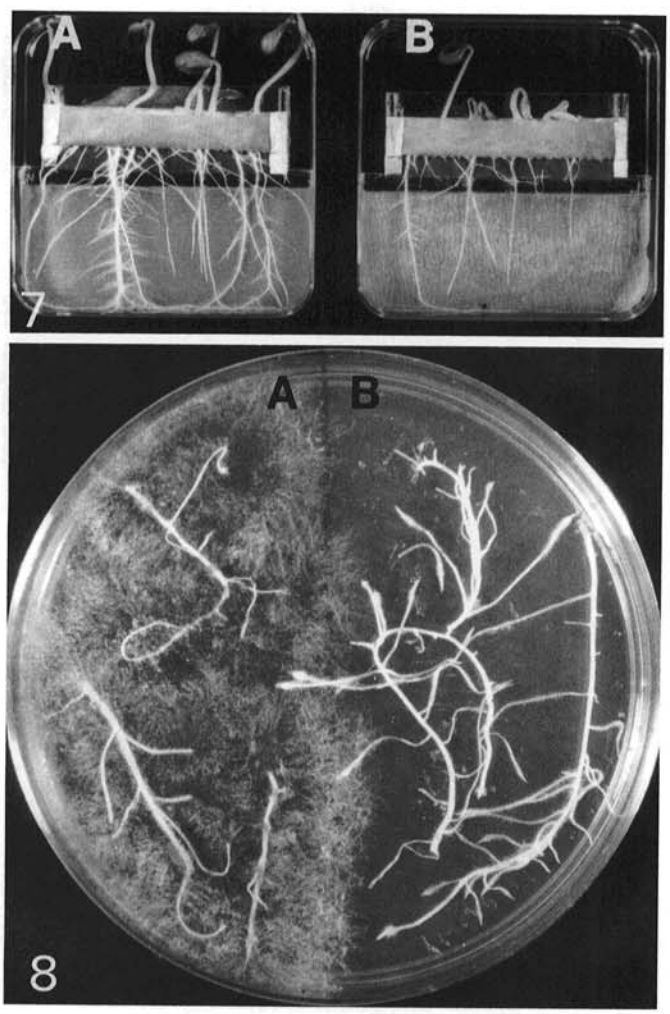
Plant source of bacteria	Strains tested (no.)	Strains (no.) positive for:	
		Colonization[a]	Inhibition[b]
Crucifer seeds	4	3	1
Pepper seeds	3	1	0
Wheat seeds	10	9	3
Apple blossoms	11	11	8
Total	28	24	12

[a] Tomato seeds amended with test strains were germinated for 60 hr and grown in seedling bioassay chambers. After 4 days, roots were excised and plated on nutrient glucose agar. Growth of test strains along roots was observed after incubation.

[b] Tomato seeds treated with test strains were inoculated with isolate K-60-STR^r and grown in seedling bioassay chambers. After 4 days, the distal 10-mm of three roots were excised, shaken in 1 ml of saline, diluted, and assayed on nutrient glucose agar containing 100 μg of streptomycin per milliliter. Numbered colonies of isolate K-60-STR^r were compared for treated and control roots.

whereas use of undiluted inoculum resulted in excessive spread of fungal mycelium in the SBA chamber.

Root colonization and antagonism towards specific pathogens. Of the 122 bacteria isolated from various plant seeds, 67% colonized collard roots, but, only 16% inhibited *X. campestris* on collard roots (Table 1). Fifty-seven strains inhibited *X. campestris* in NGA inhibition spot tests, but only 20 inhibited *X. campestris* on roots. All strains inhibitory on roots were inhibitory on agar. Strain RC-125, isolated as a laboratory contaminant, resulted in complete inhibition of *X. campestris* on roots (Fig. 6B). This strain produced a smaller (3.0 mm), but definite, inhibition zone against B-24 on NGA. Growth of *P. solanacearum* was inhibited on tomato roots by 12 of 24 strains that colonized roots (Table 2). For *R. solani*, one strain, RT-81, prevented symptoms (Fig. 7A) of damping off on cucumber during the test period and four strains delayed the expression of symptoms by 1–4 days compared to controls (Fig. 7B) (Table 3). On PDA containing streptomycin, *R. solani* was easily isolated from excised roots of seedlings showing symptoms of damping-off (Fig. 8A) but little or no growth of *R. solani* resulted from roots of seedlings with delayed or no symptoms (Fig. 8B). To further investigate bacterial antagonism to *R. solani*, 41 root-colonizing strains isolated directly from cucumber roots in SBA chambers containing samples of field soil from Georgia, Florida, Oregon, Washington, Idaho, and the Philippines were tested as described. Nineteen strains had no effect and 13 strains only delayed symptoms. However, nine strains prevented damping-off during the 8-day experiment.



Figs. 7–8. 7, Antagonism of *Rhizoctonia solani* by bacteria on cucumber seedlings in seedling bioassay chamber. **A,** Seedlings with antagonistic strain RT-81. **B,** Control seedlings (with no antagonist) showing symptoms of damping off. **8,** Inhibition of *Rhizoctonia solani* by antagonistic bacteria on cucumber roots on potato-dextrose agar (PDA). **A,** *R. solani* growing profusely from excised roots of seedlings inoculated with *R. solani* only and plated on PDA containing streptomycin. **B,** No growth of *R. solani* from roots inoculated with *R. solani* and antagonistic strain RT-81.

DISCUSSION

The executive committee of the first National Interdisciplinary Biological Control Conference stated: "...identifying and detecting biological control agents was one of the top priority items in 13 of the 15 workshops" (3). Our SBA chamber provides a means to help attain this goal.

The chamber is based on Leben's (14,15) demonstration that certain bacteria migrate from seeds to roots. It allows the use of an appropriate selective medium to visualize the presence of the pathogen and/or the antagonist on the roots. However, care must be taken to choose a medium that does not inhibit growth of the root. If such a medium is not available, water agar covered with absorbent paper permits excising and assaying the root for the presence of the pathogen and antagonist. To avoid antagonism on agar, the pathogen should be enumerated on a selective medium. Alternatively, a strain of the pathogen marked for antibiotic resistance can be used for sensitive recoveries. The presence of a sterile, dry, glass-rod barrier that separates the seeds from the agar helps ensure aseptic conditions. In addition, the glass rod apparently helps prevent bacteria from migrating along a film of moisture between the root and the agar. This apparent distinction between active and passive movement should receive further attention. Our data do not prove the prevention of passive movement, however, the failure to isolate most soil bacteria from roots in the SBA chamber suggests that it may be so. In addition, the pouch that holds the seeds enables use of field soil to determine the ability of antagonists to compete initially with natural soil flora, even though the amount of soil is small. Thus, root colonizers can be isolated directly from soil or from washings of plant parts (18).

An important requirement when testing pure cultures of pathogens or antagonists for root colonization is that seeds used to raise seedlings in the SBA chamber should be as bacteria-free as possible. Seeds also should be free of any chemical amendments. Tests can be run later to determine compatibility with chemical seed treatments. Seedborne fungi were not a problem in the normal 8-day test period.

A disadvantage of the SBA chamber is the time required to prepare the pouches. However, this is offset by not having to screen noncolonizers in greenhouse tests. For example, antagonism of single strains toward *X. campestris* can be determined within 5–6 days.

Most bacteria isolated from various seeds and roots colonized tomato roots; however, this was not true for collard roots. Our results indicate that colonization and antagonism by strains on roots is not necessarily related to their origins. Effective antagonists can be obtained from nonhost sources. Several antagonists of *X. campestris* were isolated from wheat seed, and strain RC-125,

TABLE 3. Control of damping-off of cucumber (caused by *Rhizoctonia solani* isolate AG-4) by strains of bacteria isolated from seeds

Origin of bacterial strains	Strains tested (no.)	No. of strains	
		Delaying symptoms by 1–4 days[a]	Preventing symptoms[b]
Crucifer seeds	16	1	0
Tomato seeds	5	2	1
Pepper seeds	3	0	0
Wheat seeds	11	1	0
Total	35	4	1

[a] Symptoms on at least five of six plants tested per strain in seedling bioassay chambers were delayed by 1–4 days after control plants had died.

[b] At least five of six plants in seedling bioassay chamber appeared to be healthy throughout the 8-day test period. Numbers in parenthesis are strain designations.

which was isolated as a laboratory contaminant, was strongly inhibitory toward B-24. A similar observation was reported by Pusey and Wilson (17).

All the strains antagonistic on collard roots inhibited *X. campestris* on NGA, however, the magnitude of inhibition on agar was not related to antagonism on roots. For example, strain RC-125 produced relatively small (3-mm) zones of inhibition on agar, but it was highly antagonistic in the SBA chamber. On the other hand, some strains of fluorescent pseudomonads produced 8–16 mm zones on agar but were less effective in the SBA chamber. Similarly, *Bacillus* strain NC-1 produced 22-mm zones of inhibition against *X. campestris* on NGA but failed to demonstrate antagonism on roots.

The failure of *R. solani* to grow from roots of protected seedlings of cucumber onto PDA containing streptomycin suggests that inhibition of the pathogen occurred on the roots. Elimination of associated bacterial growth by streptomycin confirms such inhibition. It is not known whether the absence of the fungus is due to competition or production of a fungitoxic substance.

The SBA chamber might be a useful tool for isolating pathogens from seeds and soil and for determining the role played by bacteria in stimulating seedling vigor. Seed pathogens borne internally often are easily isolated on roots. For example, we isolated *X. malvacearum* from fumigated cotton seed, *Phoma* sp. from surface-disinfested sugarbeet seed, and *X. transluscens* and *Alternaria* sp. from wheat seed (*unpublished*). We also isolated pathogenic fungi from several soil samples by using germinated tomato and cucumber seeds as bait. Some bacteria isolated from seed, when applied in pure culture in SBA chamber, can damage seedlings. Symptoms such as reduced germination, decay of partially germinated seeds, root collapse, general growth retardation, chlorosis, and purpling of cotyledons and hypocotyls were noticed on germinating collard seeds infested with strains RC-13, 18, 109, 110, and 114 isolated from crucifer seeds (19).

Perhaps the most valuable application of the SBA chamber might be to determine the compatibility of an antagonist with the microfloras of field soils. Soil samples could be sent to the laboratory and different antagonistic strains could be tested for compatibility. The ease of manipulating inoculum and environmental conditions such as temperature and nutrients in the SBA chamber could help determine optimum application times and concentration of antagonists. Furthermore, our SBA chamber offers a method for rapid screening of genetically-engineered antagonists. Genes for survival on roots, for antibiotic production, for resistance to other antagonistic organisms (and soil extracts) could be identified and gene pools established for constructing more versatile and effective biocontrol agents.

LITERATURE CITED

1. Backman, P. A., and Rodriguez-Kabana, R. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65:81821.
2. Baker, K. F., and Cook, R. J., eds., 1974. Biological Control of Plant Pathogens. Freeman, San Francisco, CA. 433 pp.
3. Battenfield, S. L., ed. 1983. Pages 2–4 in: Proceedings of the National Interdisciplinary Biological Control Conference. 15-17 February 1983, Las Vegas, Nevada. Dept. of Entomology, East Lansing, MI. 107 pp.
4. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
5. Chakravarti, B. P., Leben, C., and Daft, G. C. 1972. Numbers and antagonistic properties of bacteria from buds of field-grown soybean plants. Can. J. Microbiol. 18:696-698.
6. Gemrich, E. G., II, and Vandestreek, E. 1980. Effectiveness of *Bacillus uniflagellatus* in controlling plant diseases. Plant Dis. 64:382-384.
7. Johnson, L. F., and Curl, E. A. 1972. Methods for Research on the Ecology of Soilborne Plant Pathogens. Burgess Publishing Co., Minneapolis, MN. 247 pp.
8. Kempe, J., and Sequeira, L. 1983. Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. Plant Dis. 67:499-503.
9. Kerr, A., 1980. Biological control of crown gall through production of Agrocin 84. Plant Dis. 64:25-30.
10. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
11. Kleopper, J. W. 1983. Effect of seed piece inoculation with plant-growth-promoting rhizobacteria on populations of *Erwinia carotovora* on potato roots and daughter tubers. Phytopathology 73:217-219.
12. Kleopper, J. W., and Schroth, M. N. 1981. Relationship of in vitro antibiosis of plant-growth-promoting rhizobacteria to enhanced plant growth and the displacement of root microflora. Phytopatholopy 71:1020-1024.
13. Kommedahl, R., and Mew, I. C. 1975. Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65:296-300.
14. Leben, C. 1961. Microorganisms on cucumber seedlings. Phytopathology 51:553-57.
15. Leben, C. 1983. Association of *Pseudomonas syringae* pv. *lachrymans* and other bacterial pathogens with roots. Phytopathology 73:577-581.
16. Mangenot, F., and Diem, H. G. 1979. Fundamentals of biological control. Pages 207-265 in: Ecology of Root Pathogens. S. B. Krupa and Y. R. Dommergues, eds. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.
17. Pusey, P. L., and Wilson, C. L. 1983. Control of brown rot with a *Bacillus* bacterium. (Abstr.) Phytopathology 73:823.
18. Randhawa, P. S., and Schaad, N. W. 1983. A direct seedling test for selecting antagonistic bacteria. (Abstr.) Phytopathology 73:810.
19. Randhawa, P. S., and Schaad, N. W. 1984. A pathogenic fluorescent pseudomonad associated with *Brassica* seeds. (Abstr.) Phytopathology 74:757-758.
20. Randhawa, P. S., and Schaad, N. W. 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. Phytopathology 74:268-272.
21. Schaad, N. W., and Kendrick, R. 1975. A qualitative method of detecting *Xanthomonas campestris* in crucifer seed. Phytopathology 65:1034-1036.
22. Schroth, M. N., and Hancock, J. 1981. Selected topics in biological control. Annu. Rev. Microbiol 35:453-476.
23. Suslow, T. V. 1982. Role of root-colonizing bacteria in plant growth. Pages 187-223 in: Phytopathogenic Prokaryotes. Vol. I. M. S. Mount, and G. H. Lacy, eds., Academic Press, New York. 541 pp.
24. Vidaver, A. K. 1982. Biological control of plant pathogens with prokaryotes. Pages 387-397 in: Phytopathogenic Prokaryotes. Vol. II. M. S. Mount, and G. H. Lacy, eds., Academic Press, New York.
25. Weller, D. M., and Cook, R. J. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 63:463-469.