APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1993, p. 410–416
0099-2240/93/020410-07$02.00/0

Vol. 59, No. 2

Effect of Phenotypic Plasticity on Epiphytic Survival and Colonization by *Pseudomonas syringae*

MARK WILSON* AND STEVEN E. LINDOW

Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, California 94720

Received 14 September 1992/Accepted 1 December 1992

The bacterial epiphyte *Pseudomonas syringae* MF714R was cultured on agar or in broth or collected from colonized leaves; it was then inoculated onto greenhouse-grown bean plants incubated in a growth chamber at low relative humidity or in the field or onto field-grown bean plants. Cells cultured in liquid medium survived the least well after inoculation of leaf surfaces under all conditions. Cells cultured in solid medium exhibited the highest percent survival and desiccation tolerance in the growth chamber but generally survived less well in the field than did cells harvested from plants. Cells harvested from plants and inoculated onto plants in the field usually exhibited the highest percent survival, started to increase in population earlier, and reached a higher number than did cells cultured in vitro. Differences in field survival were apparently not attributable to differential UV tolerance. The observed effects of phenotypic plasticity on epiphytic survival and colonization should be considered in risk assessment studies, in studies of bacterial epidemiology, and in the use of microbial antagonists for biological pest control.

Research with plant-associated microorganisms is generally conducted with in vitro-grown laboratory cultures, which are assumed to be phenotypically representative of the naturally occurring microorganism. The possible impacts of phenotypic plasticity on the epiphytic survival and colonization potential of these microorganisms are rarely considered. Such plasticity may be particularly important in assessments of risk associated with the field application of genetically engineered microorganisms. Risk assessment studies with genetically engineered microorganisms assume that the behavior of a laboratory culture in a microcosm is a good predictor of the behavior of the same strain released into the field environment (2, 3, 12); however, this assumption is rarely tested. Evidence from the recent release of a recombinant *Pseudomonas syringae* strain (29) indicated that laboratory-cultured microorganisms were less able to survive aerosolization and survive epiphytically than were cells that had dispersed secondarily and were preadapted to the phyllosphere environment. Models developed to predict the environmental fate of genetically engineered microorganisms after field application rely on accurate estimates of microbial death rates (21, 22, 25). Although the effects of in vitro culture conditions on survival after aerosolization (31, 40) and survival on artificial surfaces (13, 39) have been examined, there have been no studies on the effects of culture conditions and preadaptation to the phyllosphere environment on epiphytic survival. Reliable estimates of the dispersal and colonization potential of genetically engineered microorganisms released into the natural environment will not be obtained if inappropriate data derived from phenotypically unrepresentative in vitro cultures are used.

Phenotypic plasticity may also affect studies on the epidemiology of plant pathogens in which the behavior of a laboratory culture is assumed to accurately reflect the behavior of the naturally occurring microorganism in the field. Phenotypic differences between laboratory cultures and cells isolated from host plant tissue in both fungal and bacterial pathogens, including *Sclerotium rolfsii* (35) and *Corynebacterium michiganense* subsp. *tesselarius* (19), have been observed. Further, pathogenicity may be greater in bacterial cells associated with host tissue than in laboratory cultures; for example, *Agrobacterium tumefaciens* associated with gall tissue caused more infections in grapevine than did the same strain cultured in the laboratory (5). Although several authors have expressed concerns that in vitro selection procedures for microbial antagonists do not select for field survival and that laboratory cultures are not as ecologically competent as the naturally occurring organism (1, 23, 37, 41), few attempts have been made to determine the effect of culture conditions on strain behavior in the field (41).

Part of the genome of plant-associated microorganisms is not expressed constitutively but requires induction by plant signals not found in the media typically used to culture these organisms in the laboratory. For example, some of the pathogenicity genes in species of the genera *Pseudomonas*, *Erwinia*, *Xanthomonas*, and *Agrobacterium* (4, 20, 26, 33, 42) and the nodulation genes in *Rhizobium trifolii* are induced by specific plant signals (17). It is apparent that the pattern of gene induction in plant-associated microorganisms is environment dependent, and hence strains cultured in different ways may be expected to exhibit considerable phenotypic plasticity. There may be considerable differences in epiphytic survival and colonization potential between laboratory cultures and cells preadapted to growth in the phyllosphere environment. Preliminary results from this laboratory indicated differences in survival between *P. syringae* cultured in vitro and the same species harvested from leaf surfaces (44). In this study we investigated the effect of phenotypic plasticity on the epiphytic survival and colonization potential of *P. syringae* in an attempt to assess the impact of this plasticity in studies of risk assessment, epidemiology, and biological control with microbial antagonists.

MATERIALS AND METHODS

Preparation of bacterial inocula. *P. syringae* MF714R was cultured on solidified King's medium B (KB), in KB broth,

* Corresponding author.

TABLE 1. Environmental conditions prevalent during field experiments[a]

Expt no.	Day				Night			
	Relative humidity (%)		Temp (°C)		Relative humidity (%)		Temp (°C)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
GH[b] A	45–90	58.4	17.8–26.7	22.8	82–100	97.0	13.9–14.4	14.3
GH B	50–97	77.4	13.3–25.6	17.5	100	100	13.3	13.3
GH C	42–100	64.6	12.2–21.1	17.6	61–99	74.4	9.4–14.4	10.4
FLD[c] A	52–100	75.4	12.2–23.3	17.6	100	100	11.1–13.3	11.7

[a] Day, 8:00 a.m. to 6:00 p.m.; night, 6:00 p.m. to 8:00 a.m.
[b] GH, greenhouse-grown plants incubated under field conditions.
[c] FLD, field-grown plants incubated under field conditions. Environmental data are not available for replicate B.

and on bean leaf surfaces. *P. syringae* MF714R was cultured on solidified KB amended with 100 μg of rifampin per ml for 24 h at 28°C. Bacterial cells were scraped from the plate and suspended in phosphate buffer (0.01 M, pH 7.0). *P. syringae* MF714R was cultured in KB broth amended with 100 μg of rifampin per ml (250-ml flasks containing 50 ml of medium) shaken at 100 rpm for 24 h at 28°C. Bacterial cells were pelleted by centrifugation and resuspended in phosphate buffer (0.01 M, pH 7.0). *P. syringae* MF714R cells preadapted to the phyllosphere environment were harvested from inoculated bean leaves. Bean plants (*Phaseolus vulgaris* cv. Bush Blue Lake 274) were grown in a greenhouse under conditions that minimized colonization by epiphytic bacterial populations. These bean plants were spray inoculated with an aqueous suspension containing 10^7 CFU of *P. syringae* MF714R per ml in phosphate buffer (0.01 M, pH 7.0). The plants were subsequently incubated in a growth chamber at 26°C and a relative humidity of 60 to 80%. Leaves were collected after 72 to 86 h, and samples of approximately 2 kg of leaf material were sonicated in chilled washing buffer (0.1 M phosphate buffer, 1.0 g of Bacto-Peptone [Difco] per liter) to dislodge the epiphytic *P. syringae* population from leaves. The bacterial suspension was filtered through Whatman no. 2 filter paper to remove plant debris and centrifuged to sediment the bacteria. Recovered viable cells of *P. syringae* MF714R constituted, on the average, approximately 30% of the total viable bacteria harvested from bean leaves.

Plant inoculation. Bacteria harvested from KB agar (solid-cultured bacteria), KB broth (liquid-cultured bacteria), or collected from leaves (plant-harvested bacteria) were suspended in phosphate buffer (0.01 M, pH 7.0) at a concentration of 10^7 MF714R cells per ml and sprayed onto bean plants at the primary leaf stage. Inocula were applied as follows: (i) to greenhouse-grown bean plants that were then incubated in a growth chamber at low relative humidity (45 to 50%), high light intensity, and a temperature of 26°C; (ii) to greenhouse-grown bean plants that were then incubated in the field; and (iii) to bean plants grown in the field. A summary of the environmental conditions experienced by the plants during the field experiments is provided in Table 1.

Experimental design and enumeration of bacteria. For each of the three incubation conditions (see above), several experiments were conducted; each experiment was considered a randomized block, which was then replicated over time. No replication of any treatment occurred within a given experiment. Twenty individual leaves were harvested at regular intervals from each treatment in each experiment. The leaves were immersed in 20 ml of washing buffer in 22-mm-diameter test tubes and sonicated for 7 min (15); appropriate dilutions were plated on KB amended with 50 μg of benomyl per ml, 100 μg of cycloheximide per ml, and 100 μg of rifampin per ml. The mean log-transformed *P. syringae* population size estimated from 20 individual leaves was determined for each treatment and sampling time for a given experiment. The time required for one-half of the cells to die after inoculation, on the average (the inoculum half-life), and the population doubling time were estimated from the slope of regressions of $\log_2$ (population size) against time in the phase of population decrease and increase, respectively, in each experiment. Analysis of variance and calculation of mean log bacterial populations were performed by using the general linear models procedure in SAS (release 6.04; SAS Institute, Cary, N.C.). Regression analysis was performed by using the Proc Reg procedure in SAS. Population estimates in some experiments were corrected by a multiplication factor that yielded similar initial cell concentrations for each treatment, since small variations in initial cell concentration of in vitro solid-cultured and liquid-cultured cells and plant-harvested cells sometimes occurred.

Measurement of UV tolerance. Bacteria harvested from KB agar or KB broth or collected from leaves were suspended in phosphate buffer (0.01 M, pH 7.0) at a concentration of 10^8 cells per ml. One milliliter of each suspension was placed in a watch glass (5 cm diameter). The watch glasses were placed on a rotary shaker (100 rpm) beneath a UV lamp (λ, 254 nm) and exposed to a flux of 10.0 erg/mm^2/s. Aliquots were removed at 10-s intervals, and appropriate dilutions were plated on KB as described previously (30).

RESULTS

Effect of culture conditions on survival and colonization of *P. syringae* on greenhouse-grown plants in the growth chamber. *P. syringae* cells cultured in liquid medium and inoculated onto greenhouse-grown plants incubated in the growth chamber exhibited the smallest percent survival, and *P. syringae* cells cultured on solid medium exhibited the highest percent survival and longest half-life. The population of liquid-cultured cells decreased on the average 260-fold in the growth chamber, in contrast to plant-harvested and solid-cultured cells, which decreased on the average only 90- and 50-fold, respectively (Fig. 1). The mean half-life of the solid-cultured cells was significantly longer ($P = 0.05$) than the mean half-life of the plant-harvested cells or the liquid-cultured cells (Table 2). In all three replicate experiments the population size of plant-harvested cells started to increase on plants earlier than did that of either liquid-cultured or solid-cultured cells (Fig. 1); however, there were no significant differences ($P = 0.05$) in the mean doubling times of the *P. syringae* populations (Table 3). In all three experiments,

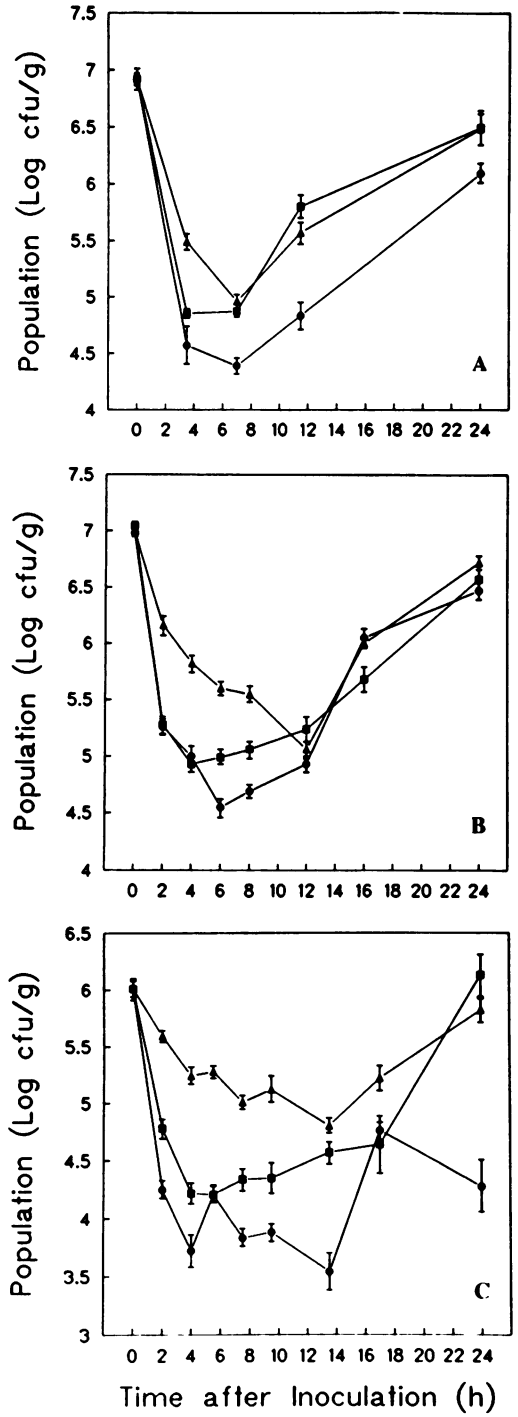

FIG. 1. Survival of *P. syringae* MF714R cultured on KB agar (▲) or in KB broth (●) or collected from bean leaf surfaces (■) on greenhouse-grown bean plants incubated in a growth chamber at low relative humidity and high light intensity in three replicate experiments (A, B, and C). Each bar represents 1 standard error of the mean.

P. syringae achieved similar population sizes after extended incubation on plants (Fig. 1).

Effect of culture conditions on survival and colonization of *P. syringae* on greenhouse-grown plants in the field. *P. syringae* cells harvested from plants and inoculated onto greenhouse-grown plants in the field exhibited a higher percent survival, started to increase earlier, and reached a higher population than did solid-cultured or liquid-cultured cells. The population of liquid-cultured cells decreased on the average 650-fold on greenhouse-grown plants in the field, compared with the populations of solid-cultured cells and plant-harvested cells, for which the average decreases were 250- and 50-fold, respectively (Fig. 2). The mean half-lives of the inocula were not significantly different ($P = 0.05$) (Table 2). In all three replicate experiments the population size of plant-harvested cells started to increase earlier and reached a higher population size than did either liquid-cultured or solid-cultured cells (Fig. 2); however, there were no significant differences ($P = 0.05$) among the *P. syringae* populations with respect to mean doubling times (Table 3).

Effect of culture conditions on survival and colonization of *P. syringae* on field-grown plants. *P. syringae* cells harvested from plants and inoculated onto field-grown plants exhibited a higher percent survival and reached a higher population than did solid-cultured or liquid-cultured cells. The population of liquid-cultured cells on inoculated plants in the field decreased 1,300-fold; solid-cultured cells and plant-harvested cells decreased 560- and 360-fold, respectively (Fig. 3). The mean half-lives of the inocula were not significantly different ($P = 0.05$) (Table 2). In both experiments, *P. syringae* derived from the plant-harvested inoculum reached the highest population size; however, there were no significant differences in the mean doubling times (Table 3).

Factors potentially involved in survival differences. Since UV light may be a significant stress experienced by epiphytic populations, we measured the ability of *P. syringae* to survive exposure to UV light in vitro; however, there were no significant differences ($P = 0.05$) in the UV tolerances of cells cultured in different ways (Fig. 4).

The survival rates of bacterial cells cultured on different solidified media were compared to determine whether prior exposure to low water potential or nutrients expected to be abundant on leaf surfaces could influence subsequent survival and colonization of the phyllosphere. Cells cultured on KB agar amended with 0.6 M sodium chloride to increase the osmotic concentration did not survive better than did cells cultured on unamended KB (data not shown). Cells cultured on water-agar containing bean leaf leachates, or on leaf diffusate medium (27) did not survive better or start to multiply earlier than did cells cultured on KB (data not shown).

DISCUSSION

Considerable differences in epiphytic survival and colonization ability were observed in *P. syringae* cells cultured under different conditions and inoculated onto plants incubated under controlled and variable field environments. Although culture age has previously been shown to affect survival of bacterial cells during in vitro desiccation (32, 38, 39), no effect on survival was demonstrated when cells derived from plant tissue were compared to cells from in vitro culture. Neither *P. syringae* pv. glycinea derived from bean leaf lesions (39) nor *Erwinia carotovora* derived from potato tubers (13) survived any better than the laboratory culture did when desiccated in vitro. The effects of phenotypic plasticity on epiphytic survival and colonization observed in this study have not been reported previously.

P. syringae populations showed remarkable ability to colonize the leaf surface under stressful environmental con-

TABLE 2. Half-lives of *P. syringae* inocula applied to plants and then incubated under different conditions[a]

Growth conditions	Half-life (min) in growth chamber[b]					Half-life (min) in field conditions						
						Greenhouse plants[c]				Field plants[d]		
	A	B	C	D	Mean	A	B	C	Mean	A	B	Mean
Agar	60.3	72.9	131.9		88.4a	94.8	47.8	47.8	63.5a	37.7	34.7	36.2a
Broth	27.0	40.1	28.8		32.0b	104.0	38.1	46.7	62.9a	31.7	28.4	30.1a
Plant	30.4	29.8	38.6	41.6	35.1b	94.9	36.9	37.1	56.3a	39.1	29.7	34.4a

[a] For each column, means followed by the same lowercase letter do not differ significantly according to the Duncan multiple-range test ($P = 0.05$). The letters in the subheadings refer to the replications of each experiment and correspond to the letters in the figures.
[b] Greenhouse-grown plants were inoculated and returned to the growth chamber.
[c] Greenhouse-grown plants were inoculated and transferred to the field.
[d] Field-grown plants were inoculated while in the field.

ditions in the absence of free moisture. Although growth of *P. syringae* on dry leaf surfaces has been observed previously (16), not all epiphytic bacterial pathogens are able to grow and maintain populations at low relative humidity (24, 43, 46). In all experiments, the size of the *P. syringae* population decreased rapidly after inoculation and exposure to environmental stress but then increased again. The drop in population size did not result from the production of viable but nonculturable cells (45). The observations are consistent with the desiccation and death of *P. syringae* cells occupying exposed sites in the phyllosphere and the multiplication of cells that had survived desiccation by occupation of protected sites (24). The processes of growth and death probably occurred concurrently, not consecutively, and the multiplication of the *P. syringae* cells in protected sites probably commenced after a variable lag phase. The percent survival and the doubling times therefore may be overestimated. The estimated doubling times of the *P. syringae* populations in the growth chamber were generally longer than the in vitro doubling times at the same temperature (47), and the doubling times of the *P. syringae* populations in the field were longer than those observed by Hirano and Upper (16) on snap-bean leaflets under similar conditions.

P. syringae cells cultured in KB broth consistently survived least well when spray inoculated onto bean leaves, although the mean half-life of viability was not significantly different from that of the plant-harvested cells. Although the liquid-cultured cells were not more sensitive than the plant-harvested cells to desiccation (as indicated by the half-life of viability), apparently a lower percentage survived desiccation, indicating that either a smaller percentage occupied protected sites or the percentage survival of plant-harvested cells was overestimated. The shorter lag phase exhibited by the plant-harvested cells could lead to an overestimation of the percent survival of these cells compared with that of the liquid-cultured cells, despite similar desiccation sensitivity. *P. syringae* cells cultured on KB agar exhibited a higher percent survival and were less sensitive to desiccation (as indicated by the longer half-life of viability) than were plant-harvested cells when inoculated onto bean leaves in the controlled conditions of the growth chamber but survived less well than plant-harvested cells in the field environment. *P. syringae* cells preadapted to the phyllosphere environment apparently survived better than in vitro cultured cells when inoculated onto bean leaves under the variable conditions of the field environment. The populations derived from preadapted cells also started to increase earlier and reached a greater size than did those derived from in vitro-cultured cells.

The phyllosphere is often considered a harsh environment subject to desiccation stress (7) and damaging UV radiation (6, 18). Several traits have been hypothesized to improve epiphytic fitness, including exopolysaccharide production, UV tolerance, and osmotolerance (28), and any one of these phenotypic traits may have been affected by the culture conditions. Although the role of exopolysaccharide in desiccation tolerance remains unproven (11, 28), *Pseudomonas* cultures incubated in sand at low water potential contained more exopolysaccharide than did those at high water potential, suggesting that, at least in the soil environment, increased exopolysaccharide production enhances survival during desiccation (36). Differences in the quality and quantity of exopolysaccharide produced by cells cultured in vitro and in vivo have been demonstrated (9, 14, 34); this may have contributed to the higher desiccation tolerance of the solid-cultured cells compared with that of the plant-harvested cells. Although UV tolerance may be a component of epiphytic fitness (28), differences in UV tolerance do not

TABLE 3. Doubling times of *P. syringae* inocula applied to plants and then incubated under different conditions[a]

Growth conditions	Doubling time (min) in growth chamber[b]					Doubling time (min) in field conditions						
						Greenhouse plants[c]				Field plants[d]		
	A	B	C	D	Mean	A	B	C	Mean	A	B	Mean
Agar	207.4	127.4	189.2		174.7a	360.7	387.6	326.3	358.2a	403.6	261.3	332.4a
Broth	181.6	125.4			153.5a	331.4	339.1	252.4	307.6a	326.6	171.1	248.8a
Plant	186.0	163.5	104.0	94.1	136.9a	275.1	423.3	268.1	322.2a	302.1	213.3	257.7a

[a] For each column, means followed by the same lowercase letter do not differ significantly according to the Duncan multiple-range test ($P = 0.05$). The letters in the subheadings refer to the replications of each experiment and correspond to the letters in the figures.
[b] Greenhouse-grown plants were inoculated and returned to the growth chamber.
[c] Greenhouse-grown plants were inoculated and transferred to the field.
[d] Field-grown plants were inoculated while in the field.

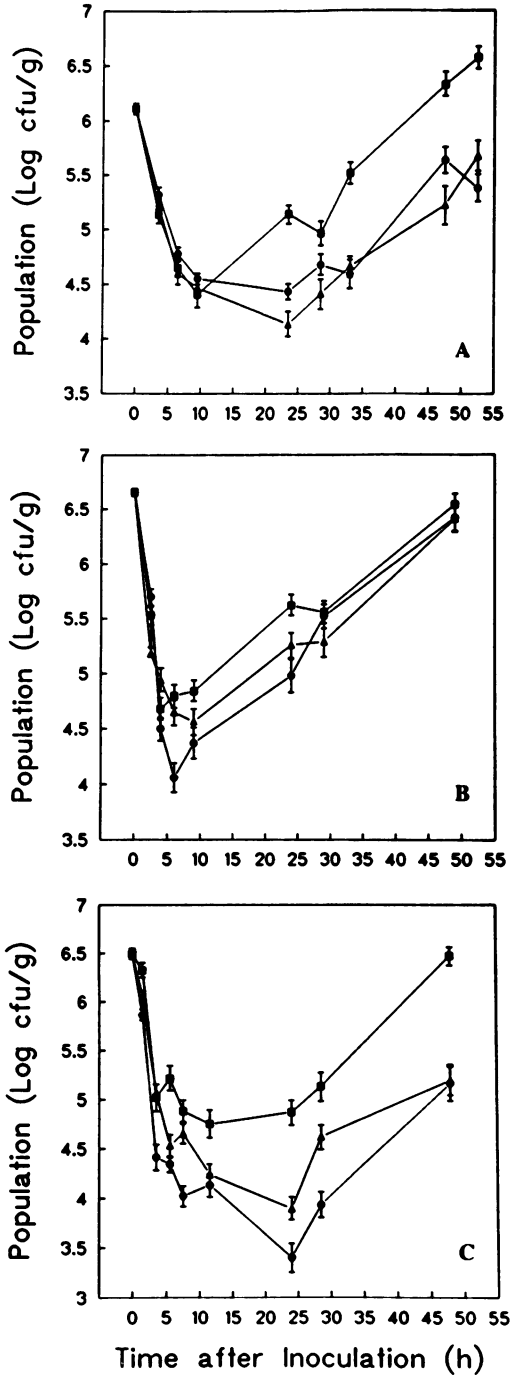

FIG. 2. Survival of *P. syringae* MF714R cultured on KB agar (▲) or in KB broth (●) or collected from bean leaf surfaces (■) on greenhouse-grown bean plants incubated in the field in three replicate experiments (A, B, and C). Each bar represents 1 standard error of the mean.

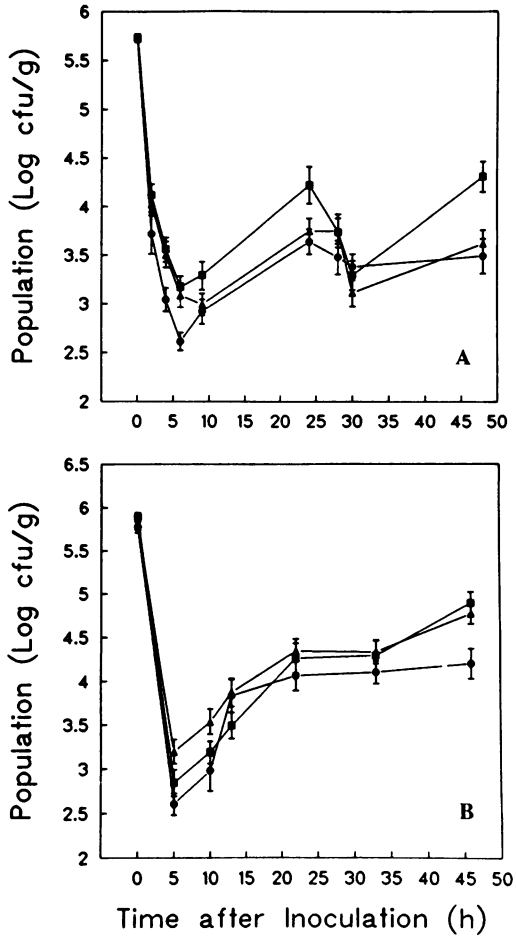

FIG. 3. Survival of *P. syringae* MF714R cultured on KB agar (▲) or in KB broth (●) or collected from bean leaf surfaces (■) on field-grown bean plants in two replicate experiments (A and B). Each bar represents 1 standard error of the mean.

appear to account for the differences in survival abilities observed in these experiments. The solid-cultured cells exhibited a small increase in UV tolerance; however, this was not significantly different from the UV tolerance of the liquid-cultured or plant-harvested cells. Tolerance of osmotic stress, produced by the concentrating of solutes in the phyllosphere at low relative humidity, may also be advantageous; however, preconditioning of cells on media with increased osmotic potential did not increase epiphytic survival of *P. syringae*. By contrast, survival of *Rhizobium meliloti* after desiccation at 0% relative humidity was improved by prior growth in media with increased osmotic potential (32).

Preadaptation to the phyllosphere environment apparently enhances the ability of *P. syringae* to survive the stressful conditions encountered in that environment and also reduces the amount of time required for adjustment to the physical and nutritional environment before multiplication. The use of solid media containing nutritional components found in leaf leachates did not induce the superior survival ability characteristic of plant-harvested cells, suggesting that these particular nutritional factors did not trigger the adaptive responses of *P. syringae*. The genes induced during preadaptation and the phenotypic traits they confer remain elusive and deserve further investigation.

These data show that culture conditions do affect phenotypic traits that are involved in epiphytic survival and colonization. Risk assessment studies, in addition to concerns about standardization of recovery and enumeration

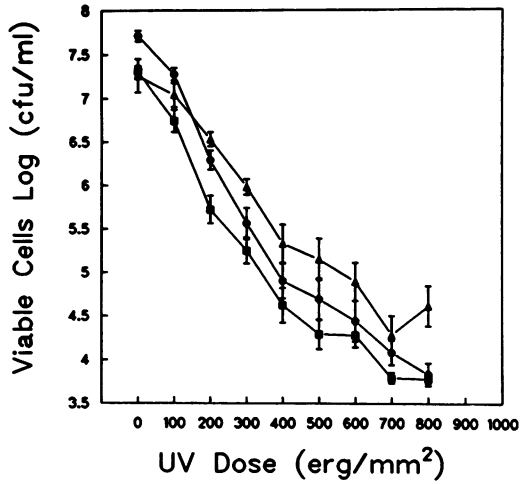

FIG. 4. Survival of *P. syringae* MF714R cultured on KB agar (▲) or in KB broth (●) or collected from bean leaf surfaces (■) after exposure to UV in vitro. Each bar represents 1 standard error of the mean.

procedures (8, 10), should direct attention to the preparation of inocula that are phenotypically representative of the organism found in association with plants. Data derived from the survival of a liquid-cultured inoculum in a microcosm are probably not good predictors of the survival of the same strain released into the field environment and dispersed secondarily from plant surfaces. Epidemiological studies should likewise note that phenotypic differences between in vitro and in vivo cultures may result in inaccurate interpretations of the epidemiology of the disease.

ACKNOWLEDGMENTS

This work was supported by grant EPA CR-815305 from the U.S. Environmental Protection Agency.

REFERENCES

1. **Andrews, J. H.** 1985. Strategies for selecting antagonistic microorganisms from the phylloplane, p. 31–44. *In* C. E. Windels and S. E. Lindow (ed.), Biological control on the phylloplane. American Phytopathological Society, Minneapolis, Minn.
2. **Armstrong, J. L.** 1991. Persistence of recombinant bacteria in microcosms, p. 495–509. *In* M. A. Levi, R. J. Seidler, and M. Rogul (ed.), Microbial ecology: principles, methods and applications. McGraw-Hill Book Co., New York.
3. **Armstrong, J. L., G. R. Knudsen, and R. J. Seidler.** 1987. Microcosm methods to assess survival of recombinant bacteria associated with plants and herbivorous insects. Curr. Microbiol. **15**:229–232.
4. **Beaulieu, C., and F. Van Gijsegem.** 1990. Identification of plant-inducible genes in *Erwinia chrysanthemi*. J. Bacteriol. **172**:1569–1575.
5. **Bishop, A. L., B. H. Katz, and T. J. Burr.** 1988. Infection of grapevines by soilborne *Agrobacterium tumefaciens* biovar 3 and population dynamics in host and non-host rhizospheres. Phytopathology **78**:945–948.
6. **Bridges, B. A.** 1976. Survival of bacteria following exposure to ultra-violet and ionizing radiations, p. 183–208. *In* T. R. G. Gray and J. R. Postgate (ed.), The survival of vegetative microbes. Cambridge University Press, Cambridge.
7. **Burrage, S. W.** 1976. Aerial micro-climate around plant surfaces, p. 173–184. *In* C. H. Dickinson and T. F. Preece (ed.), Microbiology of aerial plant surfaces. Academic Press, Inc., London.
8. **Colwell, R. R., M. A. Levin, and N. Palleroni.** 1992. Overview: historical perspective, present status and future directions, p. 11–28. *In* M. A. Levin, R. J. Seidler, and M. Rogul (ed.), Microbial ecology: principles, methods and applications. McGraw-Hill Book Co., New York.
9. **Coplin, D. L., and D. Cook.** 1990. Molecular genetics of extracellular polysaccharide biosynthesis in vascular phytopathogenic bacteria. Mol. Plant-Microbe Interact. **3**:271–279.
10. **Donegan, K., C. Maytac, R. Seidler, and A. Porteous.** 1991. Evaluation of methods for sampling, recovery and enumeration of bacteria applied to the phylloplane. Appl. Environ. Microbiol. **57**:51–56.
11. **Dudman, W. F.** 1977. The role of surface polysaccharides in natural environments, p. 357–414. *In* I. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Plenum Press, London.
12. **Elliot, E. T., H. W. Hunt, D. E. Walter, and J. C. Moore.** 1986. Microcosms, mesocosms and ecosystems: linking the laboratory to the field, p. 472–480. *In* F. Megusar and M. Gantar (ed.), Perspectives in microbial ecology. Slovene Society for Microbiology, Ljubljana, Slovenia.
13. **Graham, D. C., C. E. Quinn, and I. A. Sells.** 1979. Survival of strains of soft rot coliform bacteria on microthreads exposed in the laboratory and in the open air. J. Appl. Bacteriol. **46**:367–376.
14. **Gross, M., and K. Rudolph.** 1987. Characterization of slimes produced by *Pseudomonas phaseolicola in vitro* and *in planta*, and the demonstration of the slime polysaccharide alginate in seven *Pseudomonas syringae* pathovars, p. 523–529. *In* E. L. Collmer, A. Collmer, R. E. Davis, and A. G. Gillaspie (ed.), Sixth International Conference on Plant Pathogenic Bacteria. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.
15. **Haefele, D., and R. Webb.** 1982. The use of ultrasound to facilitate the harvesting and quantification of epiphytic and phytopathogenic microorganisms. Phytopathology **72**:947.
16. **Hirano, S. S., and C. D. Upper.** 1989. Diel variation in population size and ice nucleation activity of *Pseudomonas syringae* on snap bean leaflets. Appl. Environ. Microbiol. **55**:623–630.
17. **Innes, R. W., P. L. Kuempel, J. Plazinski, H. Canter-Cremers, B. G. Rolfe, and M. A. Djordevic.** 1985. Plant factors induce expression of nodulation and host-range genes in *Rhizobium trifolii*. Mol. Gen. Genet. **201**:426–432.
18. **Jagger, J.** 1981. Near-UV radiation effects on micro-organisms. Photochem. Photobiol. **34**:761–768.
19. **Joshi, B., and A. K. Vidaver.** 1984. Evaluation of cellular protein profiles of *Corynebacterium michiganense* ssp. *nebraskense* and *Corynebacterium michiganense* ssp. *tessellarius* from infected plants. Phytopathology **74**:879–880.
20. **Kamoun, S., and C. I. Kado.** 1990. A plant-inducible gene of *Xanthomonas campestris* pv. *campestris* encodes an exocellular component required for growth in the host and hypersensitivity on non-hosts. J. Bacteriol. **172**:5165–5172.
21. **Knudsen, G. R.** 1989. Model to predict aerial dispersal of bacteria during environmental release. Appl. Environ. Microbiol. **55**:2641–2647.
22. **Knudsen, G. R.** 1991. Models for the survival of bacteria applied to the foliage of crop plants, p. 191–216. *In* C. J. Hurst (ed.), Modelling the environmental fate of microorganisms. American Society for Microbiology, Washington, D.C.
23. **Knudsen, G. R., and H. W. Spurr.** 1988. Management of bacterial populations for foliar disease biocontrol, p. 83–92. *In* K. G. Mukerji and K. L. Garg (ed.), Biocontrol of plant diseases. CRC Press, Inc., Boca Raton, Fla.
24. **Leben, C.** 1988. Relative humidity and the survival of epiphytic bacteria with buds and leaves of cucumber plants. Phytopathology **78**:179–185.
25. **Lighthart, B., and A. S. Frisch.** 1976. Estimation of viable airborne microbes downwind from a point source. Appl. Environ. Microbiol. **31**:700–704.
26. **Lindgren, P. B., R. Frederick, A. G. Govindarajan, N. J. Panopoulos, B. J. Staskawicz, and S. E. Lindow.** 1989. An ice nucleation reporter system: identification of inducible pathogenicity genes in *Pseudomonas syringae* pv. *phaseolicola*. EMBO J. **8**:1291–1301.

27. **Lindow, S. E.** 1988. Lack of correlation of *in vitro* antibiosis with antagonism of ice nucleation active bacteria on leaf surfaces by non-ice nucleation active bacteria. Phytopathology **78:**444–450.
28. **Lindow, S. E.** 1992. Determinants of epiphytic fitness in bacteria, p. 295–314. *In* J. H. Andrews and S. S. Hirano (ed.), Microbial ecology of leaves. Springer-Verlag, New York.
29. **Lindow, S. E., G. R. Knudsen, R. J. Seidler, M. V. Walter, V. W. Lambou, P. S. Amy, D. Schmedding, V. Prince, and S. C. Hern.** 1988. Aerial dispersal and epiphytic survival of *Pseudomonas syringae* during a pre-test for release of genetically engineered strains into the environment. Appl. Environ. Microbiol. **54:**1557–1563.
30. **Loper, J. E., and S. E. Lindow.** 1987. Lack of evidence for *in situ* fluorescent pigment production by *Pseudomonas syringae* pv. *syringae* on bean leaf surfaces. Phytopathology **77:**1449–1454.
31. **Marthi, B., V. P. Fieland, M. Walter, and R. J. Seidler.** 1990. Survival of bacteria during aerosolization. Appl. Environ. Microbiol. **56:**3463–3467.
32. **Mary, P., D. Ochin, and R. Toilliez.** 1986. Growth status of rhizobia in relation to their tolerance to low water activities and desiccation stresses. Soil Biol. Biochem. **18:**179–184.
33. **Mo, Y.-Y., and D. C. Gross.** 1991. Expression *in vitro* and during plant pathogenesis of the *syr*B gene required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. Mol. Plant-Microbe Interact. **4:**28–36.
34. **Osman, S. F., W. F. Fett, and M. L. Fishman.** 1986. Exopolysaccharides of the phytopathogen *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. **166:**66–71.
35. **Punja, Z. K., S. F. Jenkins, and R. G. Grogan.** 1984. Effect of volatile compounds, nutrients, and source of sclerotia on eruptive sclerotial germination of *Sclerotium rolfsii*. Phytopathology **74:**1290–1295.
36. **Roberson, E. B., and M. K. Firestone.** 1992. Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. Appl. Environ. Microbiol. **58:**1284–1291.
37. **Schroth, M. N., J. E. Loper, and D. C. Hildebrand.** 1984. Bacteria as biocontrol agents of plant disease, p. 362–368. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
38. **Skaliy, P., and R. G. Eagon.** 1972. Effect of physiological age and state on survival of desiccated *Pseudomonas aeruginosa*. Appl. Microbiol. **24:**763–767.
39. **Sleesman, J. P., and C. Leben.** 1976. Bacterial desiccation: effects of temperature, relative humidity and culture age on survival. Phytopathology **66:**1334–1338.
40. **Southey, R. F. W., and G. J. Harper.** 1971. The survival of airborne *Erwinia amylovora* in airborne particles: tests in the laboratory and in the open air. J. Appl. Bacteriol. **34:**547–556.
41. **Spurr, H. W.** 1981. Experiments on foliar disease control using bacterial antagonists, p. 369–381. *In* J. P. Blakeman (ed.), Microbial ecology of the phylloplane. Academic Press, Inc., London.
42. **Stachel, S. E., E. W. Nester, and P. C. Zambryski.** 1986. A plant cell factor induces *Agrobacterium tumefaciens vir* gene expression. Proc. Natl. Acad. Sci. USA **83:**379–383.
43. **Timmer, L. W., J. J. Marois, and D. Anchor.** 1987. Growth and survival of xanthomonads under conditions nonconducive to disease development. Phytopathology **77:**1341–1345.
44. **Wilson, M., and S. E. Lindow.** 1990. Phenotypic plasticity affecting survival in *Pseudomonas syringae*. Phytopathology **80:**1058.
45. **Wilson, M., and S. E. Lindow.** 1992. Relationship of total viable and culturable cells in epiphytic populations of *Pseudomonas syringae*. Appl. Environ. Microbiol. **58:**3908–3913.
46. **Young, J. M.** 1978. Survival of bacteria on *Prunus* leaves, p. 779–786. *In* Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria, vol. II. Station de Pathologie Végétale et Phytobacteriologie, Institut National de la Recherche Agronomique, Angers, France.
47. **Young, J. M., R. C. Luketina, and A. M. Marshall.** 1977. The effects of temperature on growth *in vitro* of *Pseudomonas syringae* and *Xanthomonas pruni*. J. Appl. Bacteriol. **42:**345–354.