APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1994, p. 4468–4477
0099-2240/94/$04.00+0

Vol. 60, No. 12

Coexistence among Epiphytic Bacterial Populations Mediated through Nutritional Resource Partitioning

MARK WILSON* AND STEVEN E. LINDOW

Department of Environmental Science, Policy and Management, Division of Entomology, and Plant and Soil Microbiology, University of California, Berkeley, California 94720

Received 1 July 1994/Accepted 4 October 1994

The levels of coexistence between *Pseudomonas syringae* and various nonpathogenic epiphytic species in the phyllosphere of beans (*Phaseolus vulgaris*) were assessed by using replacement series. The epiphytic species *Pseudomonas fluorescens*, *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, and *Methylobacterium organophilum* were all capable of exhibiting higher levels of coexistence with *P. syringae* than was observed with a near-isogenic *P. syringae* strain pair. The ecological similarity of the epiphytes was estimated with niche overlap indices derived from in vitro carbon source utilization profiles. The level of coexistence of the epiphytes was inversely correlated with the ecological similarity of the strains. Hence, the level of coexistence between the epiphytes was proportional to the degree of niche differentiation, defined as the ability to utilize carbon sources not utilized by a competing strain. Comparisons of utilization profiles for groups of carbon sources (amino acids, organic acids, and carbohydrates) indicated the types of carbon sources for which the strains likely competed in the bean phyllosphere. *P. fluorescens* and *P. syringae* strains probably competed for most carbon sources. *S. maltophilia* and *M. organophilum* strains probably competed with *P. syringae* for most organic acids but few amino acids or carbohydrates. *P. agglomerans* strains probably competed with *P. syringae* for most amino acids and organic acids but few carbohydrates. A variable level of coexistence observed between *P. agglomerans* and *P. syringae* probably reflected the variability in abundance in the bean phyllosphere of the carbohydrates that *P. agglomerans* utilized exclusively.

Leaf surfaces of plants in terrestrial ecosystems are colonized by epiphytic communities of bacteria, yeasts, and filamentous fungi. Many factors have been suggested to be involved in determining the species composition of such communities, including availability of immigrant inoculum (30, 31, 35), host plant species (48), host plant phenology or leaf age (7), leaf position (3), and physical environmental conditions (48). The dynamics of individual populations within the epiphytic community are determined by rates of immigration, emigration, growth, and death (27, 30). A community, however, is more than an assemblage of independent populations and may be defined by the presence of interactions between its individual components. Evidence for such interactions in the epiphytic microbial community is plentiful and originates primarily from the literature on the biological control of phytopathogenic bacteria and fungi, and it includes antibiosis (24), hyperparasitism (33), and nutrient competition (2, 40).

The phyllosphere is generally considered to be a nutrient-limited environment (2). Nutrients, including carbohydrates, organic acids, and amino acids, are leached or exuded from the leaf interior into the phyllosphere. The quantities of nutrients leached or exuded from the leaf, however, are affected by leaf age, leaf physiological status, and the presence of tissue damage (13, 21, 25, 44, 51–53). Suggestions that the epiphytic community is nutrient limited come from studies in which exogenous applications of nutrients to the phyllosphere have resulted in increases in epiphytic population sizes (5, 17, 23). Populations of the epiphytic bacterium *Pseudomonas syringae* were shown to be more limited by the availability of carbon than by the availability of nitrogen (56). Competition for limiting nutritional resources has been reported between bacteria and germinating fungal spores (9, 10, 18, 42), between yeasts (8, 22), and between yeasts and phytopathogenic fungi (17, 23).

The de Wit replacement series (26) has been used extensively in plant ecology to study the nature of competitive interactions between plant species. In this substitutive design, two species are planted in mixtures of various proportions but at a constant total density and the yield of each species is determined at harvest (26). Such studies have provided important insights into niche differentiation and differential resource utilization among plant species. In microbial ecology the replacement series has been used to study the competitive interactions between phytopathogenic fungi (1) and between epiphytic bacteria (56). We previously used the replacement series to demonstrate competition for limiting nutritional resources between epiphytic *P. syringae* strains in the potato phyllosphere (56). The epiphytic *P. syringae* strains were all isolated from healthy potato leaves at one geographic location, were ecologically similar (as estimated from in vitro carbon source utilization profiles), and competed for limited carbon in the phyllosphere. These strains therefore exhibited low levels of coexistence in replacement series experiments. In a study of *P. syringae* strains isolated from diverse hosts and geographic areas, however, levels of coexistence were strain pair specific, possibly indicating ecological niche differentiation (32). On potato leaves, the epiphyte *Pseudomonas fluorescens* A506 exhibited a high level of coexistence with *P. syringae* in replacement series experiments (56). This was suggested to result from nutritional resource partitioning, or the ability of *P. fluorescens* A506 to utilize abundant carbon sources in the potato phyllosphere that were not utilized by *P. syringae* (56).

Epiphytic bacterial communities in the phyllosphere consist of diverse genera (4, 15, 19, 54) exhibiting diverse nutrient utilization patterns (19, 45, 46). Morris and Rouse (46) deter-

* Corresponding author. Mailing address: Department of Plant Pathology, 209 Life Sciences Building, Auburn University, Auburn, AL 36849-5409. Phone: (205) 844-1956. Fax: (205) 844-1947. Electronic mail address: mwilson@ag.auburn.edu.

mined the nutrient utilization profiles of randomly selected epiphytic bacterial strains from individual snap bean leaflets. While some leaflets supported epiphytic communities with numerous different nutrient utilization patterns, other leaflets supported communities with only a few different nutrient utilization patterns. The occurrence of niche differentiation between *P. fluorescens* and *P. syringae* (56) suggests that the coexistence of populations in the communities on snap beans described by Morris and Rouse (46) was mediated through nutritional resource partitioning between strains with different nutrient utilization patterns. While coexistence between community members can be mediated through mechanisms other than niche differentiation, such as temporal or spatial separation or patchiness of the environment, coexistence through niche differentiation has been demonstrated to occur in both plant (20) and insect (47) communities.

In this study we tested the hypotheses that coexistence among epiphytic bacterial populations can be mediated through nutritional resource partitioning, that the level of coexistence is proportional to ecological niche differentiation in nutritional resource utilization, and that competition for limited quantities of carbon sources is one factor determining the composition of epiphytic communities.

MATERIALS AND METHODS

Bacterial strains. All of the strains used in this study were isolated from healthy plant material and were nonpathogenic on beans (*Phaseolus vulgaris*). The origin and biochemical and ecological characteristics of *P. syringae* TLP2 and Cit7 have been described previously (36), as has the construction of the Ice^- derivatives of these strains, TLP2del1 (36) and Cit7::*xyl*E (11), respectively. The origin and characteristics of *P. fluorescens* A506 have also been described previously (55). *Pantoea agglomerans* (*Erwinia herbicola*) 299R was isolated from a healthy pear leaf (*Pyrus communis*). *P. agglomerans* (*E. herbicola*) WHL9 was isolated from a healthy hawthorn leaf (*Crataegus monogyna*). *Stenotrophomonas maltophilia* (*Xanthomonas maltophilia*) BP1 was isolated from a healthy potato leaf (*Solanum tuberosum*) in Berkeley, Calif. *Methylobacterium organophilum* SH1PK was isolated from a healthy bean leaf (*P. vulgaris*) in Madison, Wis., and was provided by S. S. Hirano.

Preparation of bacterial inocula and plant inoculation. Bacterial strains were cultured on King's medium B (KB) (29) for 18 h at 28°C. Bacterial cells were scraped from the plate and suspended in phosphate buffer (0.01 M, pH 7.0). The cell suspensions were adjusted turbidimetrically to the appropriate concentration. Replacement series experiments were conducted as described previously (56), with minor modification. Appropriate volumes of the cell suspensions were combined in six different proportions (strain A to strain B, 0:1, 0.2:0.8, 0.4:0.6, 0.6:0.4, 0.8:0.2, and 1:0) at a constant total concentration of 10^6 CFU/ml. Five replicate pots, each containing 10 bean plants (*P. vulgaris* cv. Bush Blue Lake 274), were sprayed with the inoculum mixtures. The pots were covered with plastic bags to maintain a high relative humidity, randomized within the growth chamber, and incubated for 72 h at 26°C. All replacement series experiments were repeated at least twice.

Enumeration of bacterial populations. Twenty leaves were collected randomly from each treatment, and individual leaves were placed in 20 ml of sterile washing buffer (0.1 M potassium phosphate buffer containing 0.1% Bacto Peptone, pH 7.0) in a glass tube. The tubes were sonicated in an ultrasonic cleaning bath for 7 min to dislodge the epiphytic microbial populations and vortexed briefly to suspend the cells. Serial dilutions of leaf washings were plated on KB amended with 100 μg of cycloheximide per ml and 50 μg of benomyl (Benlate; Du Pont) per ml in addition to a strain-specific selective antibiotic. *P. syringae* Cit7 and Cit7::*xyl*E were enumerated on KB amended with 100 μg of rifampin per ml. *P. syringae* Cit7::*xyl*E was distinguished from Cit7 by its yellow coloration when treated with catechol as described previously (56). *P. syringae* TLP2del1, *P. fluorescens* A506, and *P. agglomerans* 299R were enumerated on KB amended with 100 μg of rifampin per ml. These strains were distinguished from *P. syringae* TLP2del1 in coinoculations on the basis of distinctive colony morphologies. No in vitro inhibition which could have affected colony enumeration was exhibited by any of these strains on KB. *P. agglomerans* WHL9 and *M. organophilum* SH1PK were enumerated on KB amended with 50 μg of nalidixic acid per ml. *S. maltophilia* BP1 was enumerated on KB amended with 30 μg of erythromycin per ml. Epiphytic bacterial population sizes were expressed as CFU per gram (fresh weight) of leaf tissue. The mean $\log_{10}$-transformed population size of each strain pair was estimated from 20 individual leaves for each treatment. In replacement series experiments, the arithmetic back-transformed mean of the $\log_{10}$-transformed population size and the arithmetic back-transformed mean of the $\log_{10}$-transformed total population size were plotted against the inoculum proportion.

In a replacement series between two strains which compete equally for all the same limiting resources, there is a linear relationship between population size and inoculum proportion (i.e., the population size is predictable from the population size when inoculated alone and from the inoculum proportion). Further, the total population size of the strain pair is constant and is equal to the sum of the expected population sizes of the two strains (26). An increased level of coexistence of one strain with respect to the other is indicated by (i) a significant positive deviation from linearity in the relationship between population size and inoculum proportion and (ii) a total population size which is not constant over all proportions but is greater in the coinoculations than when a single strain is inoculated alone. To test the linearity of the relationship between population size and inoculum proportion, the following model was used: $\log_{10}(\text{population size}_{ij}) - \log_{10}(i) = \text{mean}_i + \text{normal error}_{ij}$, where i is the inoculum proportion and j is the leaf replicate. In this model, the population size is lognormally distributed and the relationship is linear only if all the means are equal. By analysis of variance (Proc GLM in SAS release 6.01; SAS Institute, Cary, N.C.), equality of the means was determined by an F test. The level of coexistence between *P. syringae* TLP2del1 and each epiphytic strain in the replacement series was compared statistically to the null situation (competition for all resources) by the statistical model described. The level of coexistence in replacement series was additionally quantified by calculation of the relative yield (ratio of population size when coinoculated to population size when inoculated alone) (RY) and the relative yield total (RYT) ($\text{RYT} = \text{RY}_{\text{strain 1}} + \text{RY}_{\text{strain 2}}$) for each strain pair. For those strain pairs in which there was a large differential in population sizes in the replacement series, the RY and RYT were additionally plotted against inoculum proportion. For those strain pairs exhibiting similar population sizes, however, RY and RYT were not plotted, because the graphs appear similar to those of population size plotted against inoculum proportion. A RYT of 1.0 indicates a low level of coexistence, while a RYT of greater than 1.0 indicates an increased level of coexistence and a RYT of 2.0 indicates complete coexistence (i.e., both strains achieved the same population when coinoculated as when inoculated alone) (26).

In vitro carbon source utilization profiles. The bacterial strains were tested for their ability to catabolize 154 different compounds as sole carbon sources (see Table 2). The carbon

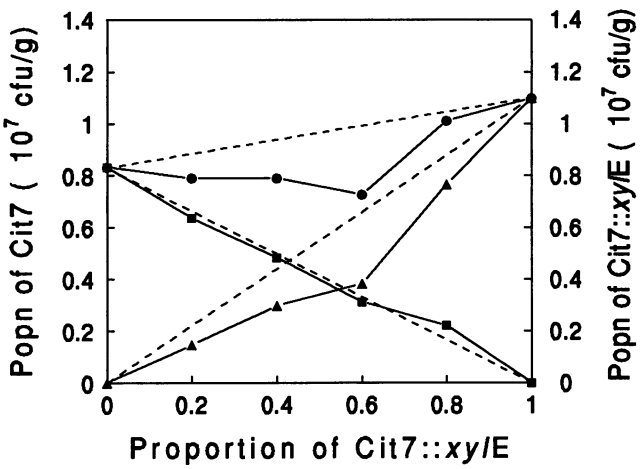

FIG. 1. Competition between *P. syringae* Cit7 and Cit7::*xyl*E. The population sizes of Cit7 (squares) and Cit7::*xyl*E (triangles) and the total population size (circles) are plotted against inoculum proportion. The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion. Population sizes of both isogenic strains deviated significantly from linearity (for Cit7, $F = 3.15$ and $P = 0.0178$; for Cit7::*xyl*E, $F = 5.09$ and $P = 0.009$).

sources were incorporated individually into minimal medium A (43) at a concentration of 10 mM. For auxotrophic *S. maltophilia* BP1, methionine, lysine, and threonine (50 μg/ml) were also added to minimal medium A. Bacterial strains were cultured on KB for 18 h at 28°C. Bacterial cells were scraped from the plate and suspended in phosphate buffer (0.01 M, pH 7.0). The cell suspensions were adjusted turbidimetrically to approximately 5×10^7 cells per ml, and 15 μl was spotted onto one plate of minimal medium A containing each carbon source. Plates were incubated for 72 h at 28°C before being scored for the presence or absence of growth.

RESULTS

Replacement series between *P. syringae* and the other epiphytic species. Replacement series experiments were conducted with the near-isogenic *P. syringae* strain pair Cit7 and Cit7::*xyl*E (see Fig. 1) and with *P. syringae* TLP2del1 and each of the epiphytic bacterial strains *P. fluorescens* A506 (see Fig. 2), *P. agglomerans* 299R (see Fig. 3), *P. agglomerans* WHL9 (see Fig. 4), *S. maltophilia* BP1 (see Fig. 5), and *M. organophilum* SH1PK (see Fig. 6). The statistical model described above was used to test the relationship between population size and inoculum proportion. The statistical test proved to be very sensitive for detection of significant deviations from linearity ($P < 0.05$); indeed most of the populations exhibited significant deviations from linearity. There was, however, generally a correlation between the F value derived from the analysis of variance and the extent of deviation from linearity, and some strains exhibited very low F values.

The near-isogenic *P. syringae* strain pair Cit7 and Cit7::*xyl*E exhibited a low level of coexistence in the replacement series on beans (Fig. 1 and Table 1). In the replacement series between *P. syringae* TLP2del1 and *P. fluorescens* A506 on beans, the population size of *P. fluorescens* A506 was significantly larger than the population size of *P. syringae* TLP2del1 when inoculated alone; hence, the RY, as well as the population size, was plotted against inoculum proportion (Fig. 2A and B, respectively). *P. fluorescens* A506 and *P. syringae* TLP2del1 both exhibited a significantly higher level of coexistence than the near-isogenic *P. syringae* strain pair (Fig. 2 and Table 1). The population sizes of *P. agglomerans* 299R and *P. syringae* TLP2del1 were similar when inoculated alone; hence, only the population size and not the RY was plotted against inoculum proportion (Fig. 3). The level of coexistence exhibited by *P. agglomerans* 299R and *P. syringae* TLP2del1 was variable between experiments conducted at different times of the year. The interaction was variously characterized by a low level of coexistence of both strains (Fig. 3A and Table 1), a high level of coexistence of both strains (Fig. 3B and Table 1), and a low level of coexistence of TLP2del1 with respect to 299R plus a high level of coexistence of 299R with respect to TLP2del1 (Fig. 3C and Table 1). The population sizes of *P. agglomerans* WHL9 and *P. syringae* TLP2del1 were also similar when inoculated alone (Fig. 4). The level of coexistence exhibited by *P. agglomerans* WHL9 and *P. syringae* TLP2del1 was also variable in different experiments and included a low level of coexistence of both strains (Fig. 4A and Table 1) and a low level of coexistence of WHL9 with respect to TLP2del1 plus a high level of coexistence of TLP2del1 with respect to WHL9 (Fig. 4B and Table 1). The population size of *S. maltophilia* BP1 was significantly larger than the population size of *P. syringae* TLP2del1 when inoculated alone; hence, the RY, as well as the population size, was plotted against inoculum proportion (Fig. 5A and B, respectively). *S. maltophilia* BP1 and *P. syringae* TLP2del1 both exhibited a high level of coexistence with respect to each other (Fig. 5B and Table 1). The population size of *P. syringae* TLP2del1 was significantly larger than the population size of *M. organophilum* SH1PK when inoculated alone; hence, the RY, as well as the population size, was plotted against inoculum proportion (Fig. 6A and B, respectively). *M. organophilum* SH1PK and *P. syringae* TLP2del1 both exhibited a high level of coexistence with respect to each other (Fig. 6B and Table 1).

TABLE 1. Levels of coexistence between *P. syringae* TLP2del1 and epiphytic species determined from replacement series experiments

Figure	Strain	Mean RY[a]	Mean RYT[b]
1	*P. syringae* Cit7	0.497	0.860
	P. syringae Cit7::*xyl*E	0.363	
2	*P. syringae* TLP2del1	0.635	1.404
	P. fluorescens A506	0.686	
3A	*P. syringae* TLP2del1	0.532	0.920
	P. agglomerans 299R	0.388	
3B	*P. syringae* TLP2del1	0.708	1.546
	P. agglomerans 299R	0.837	
3C	*P. syringae* TLP2del1	0.514	1.711
	P. agglomerans 299R	1.196	
4A	*P. syringae* TLP2del1	0.584	1.158
	P. agglomerans WHL9	0.574	
4B	*P. syringae* TLP2del1	0.807	1.294
	P. agglomerans WHL9	0.487	
5	*P. syringae* TLP2del1	0.723	1.596
	S. maltophilia BP1	0.872	
6	*P. syringae* TLP2del1	0.912	1.957
	M. organophilum SH1PK	1.045	

[a] Level of coexistence of one strain in a figure with respect to the other determined from the mean of RYs for the four inoculum proportions containing both strains.

[b] Level of coexistence of both strains in a figure determined from the mean of the RYTs for the four inoculum proportions containing both strains. A mean RY of approximately one or a mean RYT of approximately two indicates complete coexistence of one strain with the other.

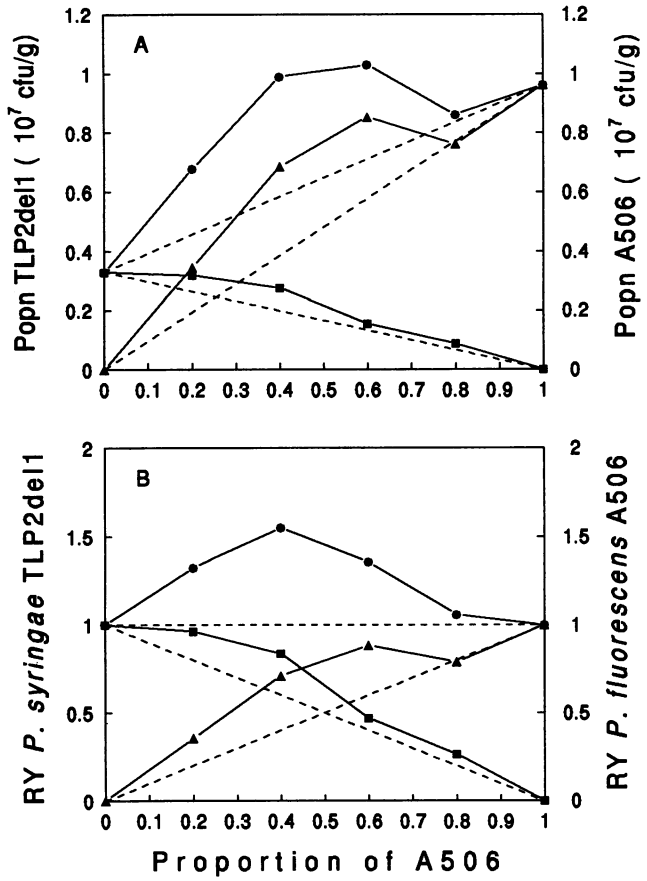

FIG. 2. Competition between *P. syringae* TLP2del1 and *P. fluorescens* A506. (A) The population sizes of TLP2del1 (squares) and A506 (triangles) and the total population size (circles) are plotted against inoculum proportion. The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion. The population size of TLP2del1 did not deviate significantly from linearity ($F = 1.08$; $P = 0.3725$). The population size of A506 deviated significantly from linearity ($F = 9.08$; $P = 0.0001$). (B) The $RY_{TLP2del1}$ (squares), RY_{A506} (triangles), and RYT (circles) are plotted against inoculum proportion.

Nutrient addition in the phyllosphere. Amendment of the phyllosphere with certain amino acids at the time of inoculation with the epiphytic strains *P. syringae* TLP2del1 and *P. agglomerans* 299R resulted in final population sizes of those strains significantly larger than sizes in the absence of the nutrient amendments (Fig. 7). In one experiment the population size of *P. syringae* TLP2del1 was significantly enhanced by the amino acids alanine, arginine, asparagine, aspartic acid, glutamic acid, histidine, phenylalanine, proline, and serine (Fig. 7A), while in another experiment (data not shown) significant enhancement was additionally observed with glutamine but not observed with phenylalanine. In vitro *P. syringae* TLP2del1 catabolized all of these amino acids except for phenylalanine as sole carbon sources. The population size of *P. agglomerans* 299R was significantly enhanced by the amino acids alanine, asparagine, aspartic acid, glutamic acid, glutamine, histidine, and proline (Fig. 7B), and in vitro *P. agglomerans* catabolized all these amino acids as sole carbon sources. With the exception of phenylalanine in one experiment, epiphytic population sizes were significantly increased

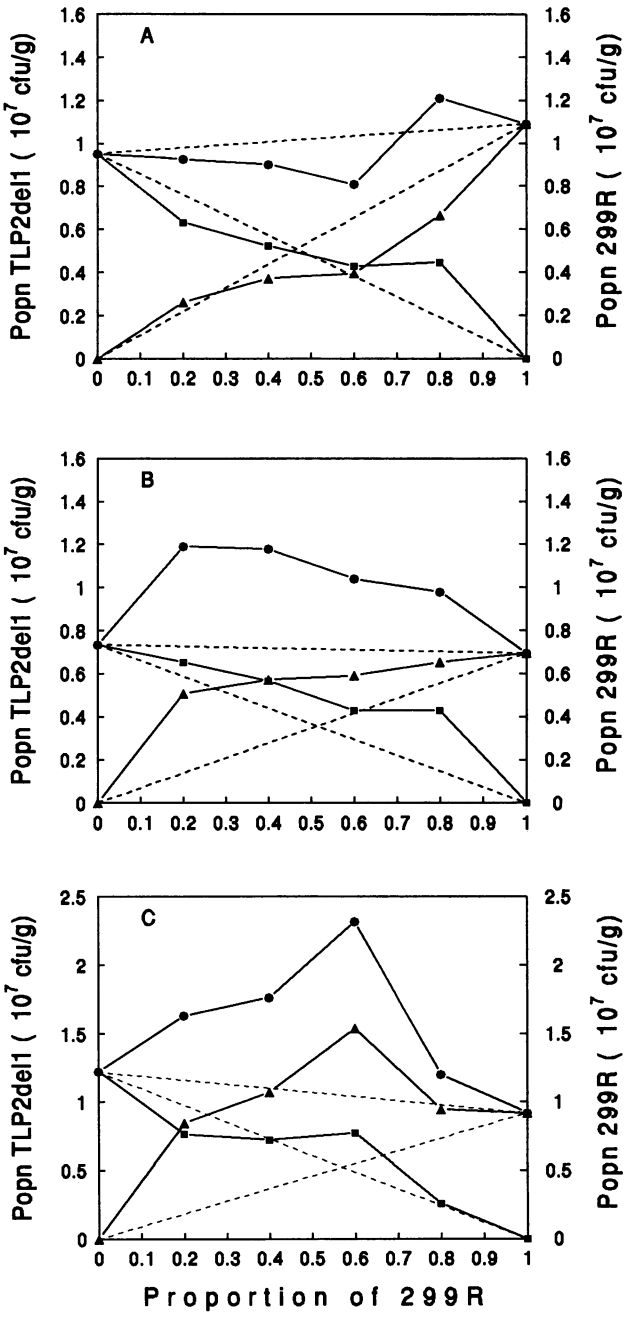

FIG. 3. Competition between *P. syringae* TLP2del1 and *P. agglomerans* 299R in three different experiments. The population sizes of *P. syringae* TLP2del1 (squares) and *P. agglomerans* 299R (triangles) and the total population size (circles) are plotted against inoculum proportion. (A) Both population sizes deviated significantly from linearity (for TLP2del1, $F = 4.65$ and $P = 0.019$; for 299R, $F = 3.69$ and $P = 0.008$). (B) Both population sizes deviated significantly from linearity (for TLP2del1, $F = 14.43$ and $P = 0.0001$; for 299R, $F = 44.32$ and $P = 0.0001$). (C) Both population sizes deviated significantly from linearity (for TLP2del1, $F = 31.87$ and $P = 0.0001$; for 299R, $F = 3.02$ and $P = 0.028$). The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion.

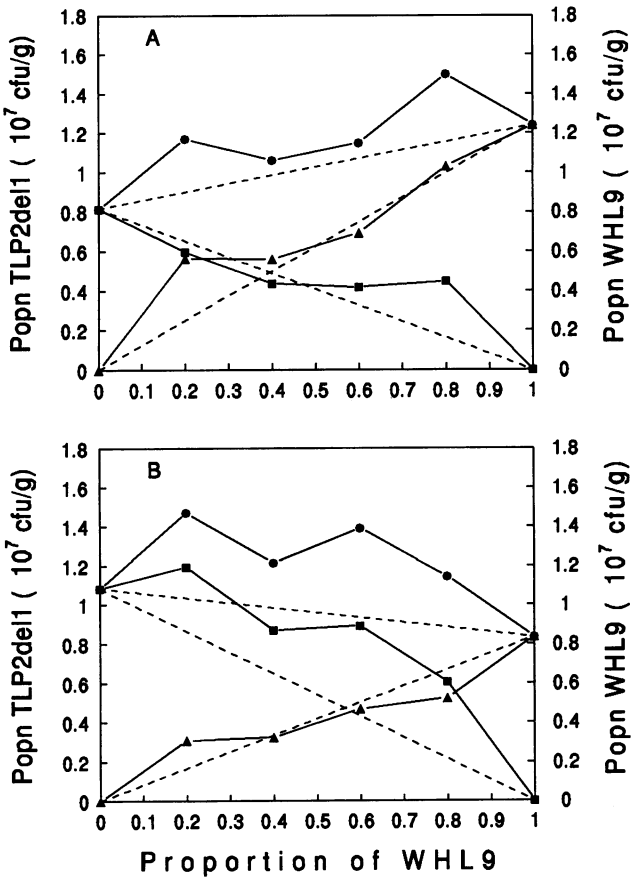

FIG. 4. Competition between *P. syringae* TLP2del1 and *P. agglomerans* WHL9 in two different experiments. The population sizes of *P. syringae* TLP2del1 (squares) and *P. agglomerans* WHL9 (triangles) and the total population size (circles) are plotted against inoculum proportion. (A) Both population sizes deviated significantly from linearity (for TLP2del1, $F = 22.84$ and $P = 0.001$; for WHL9, $F = 12.37$ and $P = 0.0001$). (B) Both population sizes deviated significantly from linearity (for TLP2del1, $F = 4.94$ and $P = 0.0012$; for WHL9, $F = 3.15$ and $P = 0.0178$). The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion.

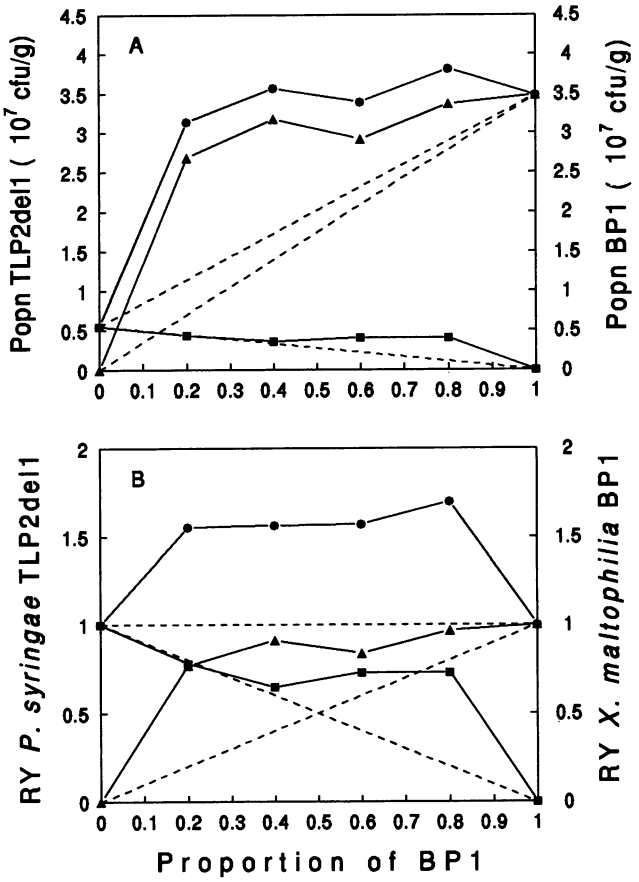

FIG. 5. Competition between *P. syringae* TLP2del1 and *S. maltophilia* BP1. (A) The population size of *P. syringae* TLP2del1 (squares), the population size of *S. maltophilia* BP1 (triangles), and the total population size (circles) are plotted against inoculum proportion. Both population sizes deviated significantly from linearity (for TLP2del1, $F = 39.52$ and $P = 0.0001$; for BP1, $F = 23.74$ and $P = 0.0001$). The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion. (B) The $RY_{TLP2del1}$ (squares), RY_{BP1} (triangles), and RYT (circles) are plotted against inoculum proportion.

only by those compounds which were catabolized in vitro as sole carbon sources, and all the amino acids that were catabolized in vitro as sole carbon sources significantly increased the population size.

Ecological similarity and coexistence. In order to estimate niche similarity of the epiphytic species in an ecologically significant resource dimension, in vitro carbon source utilization profiles were compared and niche overlap indices (NOIs) were derived (56). The epiphytes were tested for their ability to utilize each of 154 different compounds as a sole carbon source (Table 2). The NOI was defined in this study as the number of carbon sources utilized by both strains as a proportion of the total number of carbon sources utilized by the strain in question (56). NOIs were estimated for each strain in a pair (Table 3). While NOIs derived from total carbon source utilization data provided information on the degree of ecological similarity of the strains, the NOIs derived from groups of carbon sources (amino acids, organic acids, or carbohydrates) provided information on the overlap in ability to catabolize those compounds (Table 3). For example, in vitro *P. fluorescens* A506 utilized 100% of the carbohydrates ($NOI_{TLP2del1} = 1.000$), 90% of the amino acids ($NOI_{TLP2del1} = 0.900$), and 87% of the organic acids ($NOI_{TLP2del1} = 0.870$) utilized by *P. syringae* TLP2del1. *P. syringae* TLP2del1 in turn utilized 56% of the amino acids, 69% of the organic acids, and 88% of the carbohydrates utilized by *P. fluorescens* A506. In another example, *P. syringae* utilized 100% of the amino acids and 94% of the organic acids but only 41% of the carbohydrates utilized by *P. agglomerans* 299R. *P. agglomerans* 299R utilized 100% of the carbohydrates but only 65% of the organic acids and 70% of the amino acids utilized by *P. syringae*. The strain pair *S. maltophilia* BP1 and *P. syringae* TLP2del1 and the strain pair *M. organophilum* SH1PK and *P. syringae* TLP2del1 showed low NOIs for all of the carbon sources, but particularly the amino acids and carbohydrates.

The level of coexistence of each strain, the mean RY in the replacement series (Table 1), was regressed against the ecological similarity of the strains, the NOI derived from the total carbon source utilization profile (Table 3 and Fig. 8A). The level of coexistence of each strain with respect to the other was inversely correlated with the ecological similarity of each strain

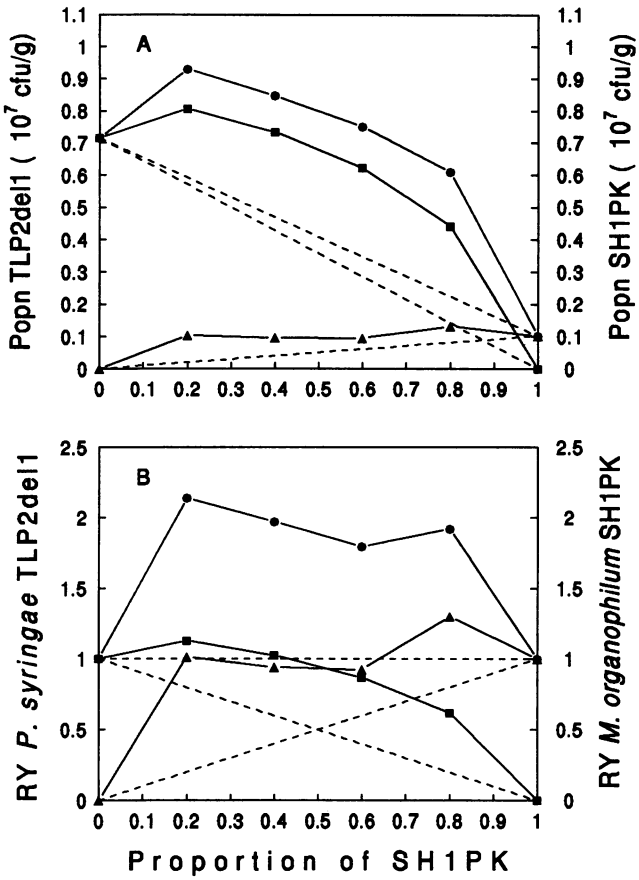

FIG. 6. Competition between *P. syringae* TLP2del1 and *M. organophilum* SH1PK. (A) The population size of *P. syringae* TLP2del1 (squares), the population size of *M. organophilum* SH1PK (triangles), and the total population size (circles) are plotted against inoculum proportion. Both population sizes deviated significantly from linearity (for TLP2del1, $F = 11.80$ and $P = 0.001$; for SH1PK, $F = 17.71$ and $P = 0.001$). The dashed lines represent the expected populations based upon the population size when inoculated alone and the inoculum proportion. (B) The $RY_{TLP2del1}$ (squares), the RY_{SH1PK} (triangles), and the RYT (circles) are plotted against inoculum proportion.

with respect to the other (Fig. 8A). The slope of the regression approximated that modeled by our theory (Fig. 8A). The level of coexistence of each strain pair, the mean RYT in the replacement series (Table 1), was plotted against the ecological similarity of the strains, the mean NOI of the two strains derived from the total carbon source utilization profile (Table 3 and Fig. 8B). The average level of coexistence of the strain pair was inversely correlated with the average ecological similarity of the strain pair (Fig. 8B). The slope of the regression approximated that modeled by our theory (Fig. 8B).

DISCUSSION

Complete coexistence of one epiphytic bacterial strain with another is defined in this study as the ability of that strain to achieve the same population size in the presence of the competing strain as in its absence. Usually, however, the level of coexistence of one strain with respect to another falls on a continuum between complete competition (populations limited by the same resources) and complete coexistence (populations limited by different resources). Further, the two strains in a pair do not necessarily exhibit the same level of coexistence with respect to each other. When the levels of coexistence between *P. syringae* TLP2del1 and the other epiphytic strains were assessed by the replacement series methodology, different levels of coexistence between the strains were observed. The epiphytic strains *P. fluorescens* A506, *P. agglomerans* 299R and WHL9, *S. maltophilia* BP1, and *M. organophilum* SH1PK all exhibited higher levels of coexistence with *P. syringae* TLP2del1 in the bean phyllosphere than was observed with the near-isogenic *P. syringae* strain pair under the same conditions. *S. maltophilia* BP1 and *P. fluorescens* A506 also exhibited a high level of coexistence with *P. syringae* TLP2del1 in the potato phyllosphere, and *S. maltophilia* exhibited a high level of coexistence with *P. syringae* Cit7del1b in both the bean and potato phyllospheres (unpublished data). This suggests that the higher level of coexistence of these epiphytic strains was not restricted to a single host or *P. syringae* strain.

The high levels of coexistence observed in these strain pairs suggests the existence of niche differentiation between *P. syringae* and the other epiphytic strains. The ecological similarity of the strains was estimated with NOIs. The intensity of competition in microbial interactions can be correlated with ecological niche overlap only if the overlap is measured in a limiting resource dimension (28, 34, 49). Epiphytic populations on greenhouse-grown plants maintained under constant environmental conditions were limited by carbon availability; hence, NOIs derived from total in vitro carbon source utilization profiles provided a good estimate of the in planta ecological similarity of the strains (56). The NOIs, and ecological similarity, of the epiphytic strains of *P. fluorescens*, *P. agglomerans*, *S. maltophilia*, and *M. organophilum* with respect to *P. syringae* were lower (ranging from 0.400 to 0.714) than the NOIs, and ecological similarity, observed previously for nonisogenic *P. syringae* strain pairs (ranging from 0.900 to 1.000) or for the near-isogenic *P. syringae* strain pair (1.00) (56). The NOIs for *P. syringae* with respect to *P. agglomerans*, *S. maltophilia*, and *M. organophilum* were also low (ranging from 0.365 to 0.654), but with respect to *P. fluorescens* the NOI was high (0.923) and comparable to the NOIs observed previously for the nonisogenic *P. syringae* strain pair (ranging from 0.900 to 1.000) (56).

The level of coexistence of the epiphytic bacterial strains in the phyllosphere was inversely correlated with the estimated ecological similarity of the strains. While Lindow (37) hypothesized that the "antagonism due to competition of one strain with another would increase proportionally to the overlap of their ecological niches," the correlation has not been tested previously because of the inability to quantify either coexistence or ecological similarity between epiphytic bacteria. The inverse correlation between level of coexistence and ecological similarity confirms the ecological significance of NOIs estimated from in vitro carbon source utilization profiles and supports the hypothesis of Lindow (37). The slight difference in the slopes of the observed and predicted regressions of the level of coexistence against ecological similarity (Fig. 8), however, suggests that either the level of coexistence was overestimated or the niche overlap was underestimated. An overestimation of the level of coexistence would suggest that a small proportion of the observed coexistence was attributable to factors other than resource partitioning. An underestimation of the NOIs could result from the extensive list of substrates used in the in vitro carbon source utilization profiles, many of which probably do not occur in the bean phyllosphere. This suggests that the usefulness of NOIs for examining epiphytic communities would be substantially improved if they were based on only those carbon sources actually present in the

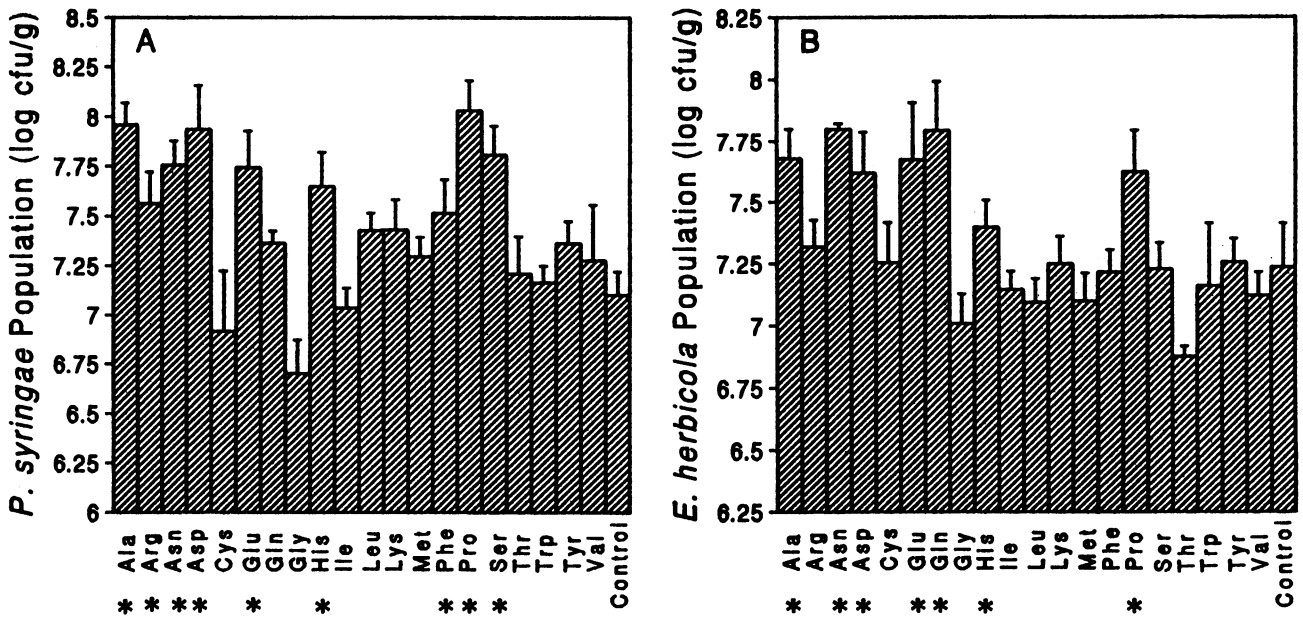

FIG. 7. Bean plants (*P. vulgaris* cv. Bush Blue Lake 274) were spray inoculated with *P. syringae* TLP2del1 or *P. agglomerans* 299R. The inoculum was supplemented with 1 of 20 different amino acids at a concentration of 2.0 g/liter. The plants were incubated in the growth chamber under constant environmental conditions of 26°C and high relative humidity. Shown are population sizes of *P. syringae* TLP2del1 (A) and *P. agglomerans* 299R (B). Bars represent 1 standard error of the mean. Asterisks indicate populations sizes which are significantly ($P = 0.05$) larger than the population size in the absence of nutrient amendment (control).

phyllosphere under examination. Further, the variability in the level of coexistence of *P. agglomerans* with *P. syringae* suggests that variation in resource availability is important in these interactions. If the abundance of individual resources (in this case carbon sources) is highly variable, NOIs which do not take account of resource abundance are of only limited usefulness (41). NOIs weighted for the abundance of the carbon sources occurring in the phyllosphere under examination should theoretically provide superior correlations with the levels of coexistence between epiphytic populations.

The inverse correlation between NOIs and the level of coexistence could be used to identify antagonists which may be effective in the preemptive exclusion of a target bacterial strain (57). A high NOI with respect to the target pest, indicating a high degree of ecological similarity, would predict that the strain would be effective in the preemptive exclusion of the target pest, by usurping a high percentage of the resources that would otherwise be available to the target pest. For example, the low NOIs for *P. agglomerans* 299R and WHL9 with respect to *P. syringae* TLP2del1 predict that *P. agglomerans* would not

TABLE 2. Compounds tested for their ability to support growth of the bacterial strains as sole carbon sources when incorporated into minimal medium A

Type	Compounds
Amino acids	D-Alanine, L-alanine, L-alanylglycine, L-arginine, L-aspartic acid, L-asparagine, citrulline, L-cysteine, L-glutamic acid, L-glutamine, L-glycine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-homoserine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, pyro-glutamic acid, D-serine, L-serine, threonine, tryptophan, valine, norvaline
Organic acids	Adipic acid, acetic acid, aconitic acid, anthranilic acid, α-aminobutyric acid, γ-aminobutyric acid, citraconic acid, citric acid, folic acid, formic acid, fumaric acid, galacturonic acid, galacturonic acid lactone, polygalacturonic acid, gentisic acid, gluconic acid, glucuronic acid, glucosaminic acid, glutaric acid, glyceric acid, glycolic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, hyroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, lactic acid, malic acid, maleic acid, malonic acid, methylpyruvic acid, monomethylsuccinic acid, mucic acid, nicotinic acid, *para*-aminobenzoic acid, pantothenic acid, pimelic acid, pipecolic acid, propionic acid, pyruvic acid, quinic acid, saccharic acid, salicylic acid, sebacic acid, shikimic acid, succinic acid, bromosuccinic acid, succinamic acid, tartaric acid, urocanic acid
Carbohydrates	Amylose, arabinose, cellobiose, cyclodextrin, dextran, dextrin, fructose, fucose, furanose, galactose, gentiobiose, glucose, glucose 1-phosphate, glucose 6-phosphate, glycogen, lactose, lactulose, maltose, mannose, melezitose, melibiose, palatinose, psicose, raffinose, rhamnose, ribose, sorbose, starch, sucrose, trehalose, xylose
Sugar alcohols	Adonitol, arabitol, dulcitol, erythritol, inositol, mannitol, sorbitol, xylitol
Amides and amines	Acetamide, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, alaninamide, glucuronamide, phenylethylamine
Alcohols	2,3-Butanediol, ethanol, 2-aminoethanol, DL-α-glycerolphosphate, glycerol, methanol
Fatty acids	Capric acid, caproic acid, caprylic acid, lauric acid, lauryl sulfate, levulinic acid, myristic acid
Miscellaneous	Betaine, DL-carnitine, choline chloride, inosine, β-methylglucoside, ornithine, putrescine, salicin, sarcosine, thymidine, Tween 40, Tween 80

TABLE 3. NOIs for epiphytic strains paired with *P. syringae*, derived from carbon source utilization data

Strain competing with *P. syringae* TLP2del1	Niche size[a]	Total compounds utilized[b]		Amino acids		Organic acids		Carbohydrates	
		$NOI_{TLP2del1}$[c]	NOI_{strain}[d]	$NOI_{TLP2del1}$	NOI_{strain}	$NOI_{TLP2del1}$	NOI_{strain}	$NOI_{TLP2del1}$	NOI_{strain}
P. fluorescens A506	77	0.923	0.623	0.900	0.562	0.870	0.690	1.000	0.875
P. agglomerans 299R	48	0.654	0.708	0.700	1.000	0.652	0.938	1.000	0.411
P. agglomerans WHL9	42	0.577	0.714	0.600	1.000	0.609	0.933	0.857	0.462
S. maltophilia BP1	43	0.365	0.442	0.700	0.583	0.348	0.615	0.286	0.222
M. organophilum SH1PK	47	0.404	0.446	0.000	0.000	0.609	0.500	0.143	0.111

[a] Per 154 carbon sources. The niche size for *P. syringae* TLP2del1 was 52.
[b] Generated by addition of 154 carbon sources individually to minimal medium A.
[c] $TNOI_{TLP2del1}$ represents the proportion of the carbon compounds utilized by TLP2del1 that were also utilized by the competing strain.
[d] NOI_{strain} represents the proportion of the carbon compounds utilized by the strain that were also utilized by TLP2del1.

be a good biological control agent for the preemptive exclusion of *P. syringae* strains, but the high NOI of *P. fluorescens* A506 predicts that strain A506 would be a good biological control agent for the preemptive exclusion of *P. syringae* strains. In greenhouse and field trials *P. agglomerans* was found to be less effective in the preemptive exclusion of Ice^+ *P. syringae* strains than other pseudomonads (12, 39). In a more recent field trial, *P. agglomerans* 299R was less effective than *P. fluorescens* A506 in reducing Ice^+ *P. syringae* populations and in preventing frost injury of pear trees (38a).

These findings provide evidence that the epiphytic bacterial populations incubated under these conditions competed for carbon in the phyllosphère and further suggest that competition for carbon was a major factor determining the composition of the epiphytic bacterial community under these conditions. It is possible, however, that under different conditions the C/N ratio of the phyllosphere would be supraoptimal for the growth of these epiphytic bacteria, rather than suboptimal as in these studies, and hence nitrogen would likely become the limiting nutritional resource. Under such conditions, the epiphytic bacterial populations would likely compete for nitrogen and the composition of the epiphytic bacterial community would then be determined by competition for nitrogen, not carbon.

The guild concept of community ecology could be usefully applied to epiphytic microbial communities. A guild is defined as a group of species that exploit the same class of resources in similar ways. In a guild, species that overlap significantly in their niche requirements are grouped without regard to their taxonomic positions (50). Epiphytic populations in the phyllosphere community could be grouped into guilds of species exhibiting a minimum similarity in the use of carbon or nitrogen sources. This could be achieved by cluster analysis techniques based on carbon or nitrogen utilization profiles. Clusters generated in this manner would be preferable to clusters based on less ecologically significant phenotypic characteristics (4, 16, 19). The minimum level of carbon or nitrogen source utilization similarity could be chosen on the basis of the desired level of coexistence of the clusters, estimated from the relationship between NOI and RY. Grouping of epiphytic populations into guilds of catabolically equivalent species would facilitate the study of complex nutritional relationships in epiphytic communities. Morris (45) grouped epiphytic bacterial isolates from snap bean leaflets into nutrient utilization

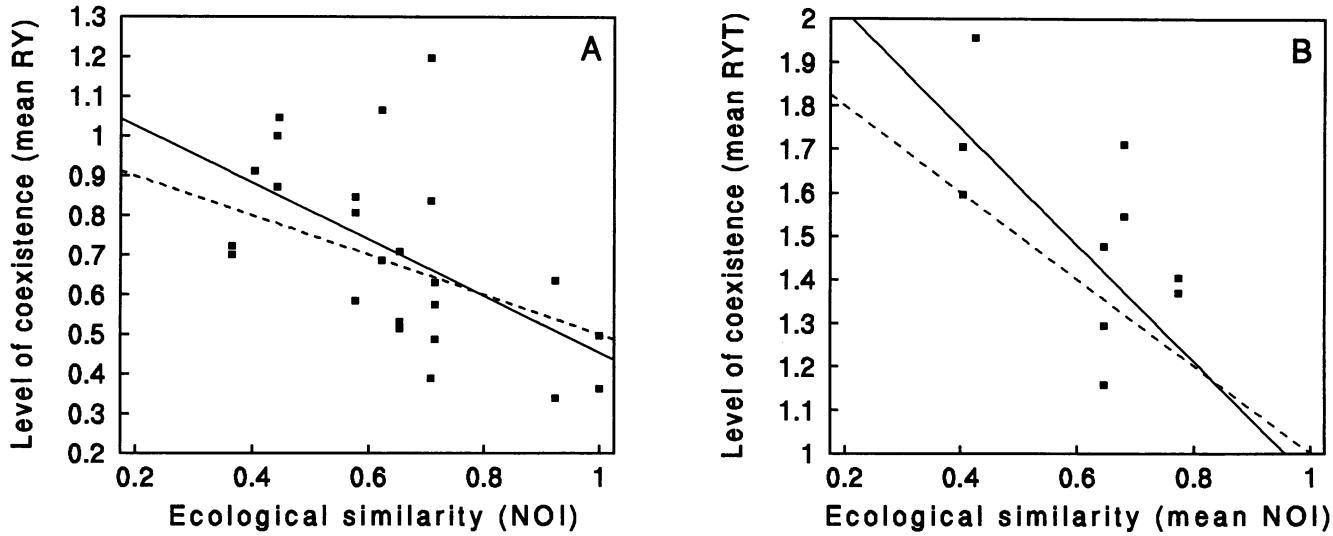

FIG. 8. Correlation between level of coexistence and ecological similarity. (A) The solid line shown represents the regression of the mean RY (Table 1) of each strain and the mean NOI (Table 3) of each strain [$P = 0.0038$; $y = (-0.714 \cdot x) + 1.167$; $R^2 = 0.322$]. The dashed line represents the predicted theoretical relationship [$y = (-0.5 \cdot x) + 1.0$] based upon an ecological similarity of 0 giving a mean RY of 1.0 and an ecological similarity of 1.0 giving a mean RY of 0.5. (B) The solid line shown represents the regression of the mean RYT (Table 1) and the mean NOI (Table 3) of the strain pair [$P = 0.0089$; $y = (-1.344 \cdot x) + 2.285$; $R^2 = 0.512$]. The dashed line represents the predicted theoretical relationship [$y = (-1.0 \cdot x) + 2.000$] based upon an ecological similarity of 0 giving a mean RYT of 2.0 and an ecological similarity of 1.0 giving a mean RYT of 1.0.

clusters based on an arbitrarily chosen average level of dissimilarity in nutrient utilization profiles which included both carbon and nitrogen sources. The ideal cluster analysis, however, would be based on utilization of either carbon or nitrogen, or whatever resource was more limiting in the phyllosphere under examination, with a level of dissimilarity selected on the basis of the desired level of coexistence of the clusters. The clusters produced in this way would have greater ecological significance than those produced by Morris (45), because the clusters would represent strains competing for similar limiting resources and not similar arbitrarily chosen resources.

The NOIs determined for the strains by using groups of carbon sources (amino acids, organic acids, and carbohydrates) provide useful information about which types of carbon sources the strains probably competed for in the bean phyllosphere. *P. fluorescens* A506 probably competed with *P. syringae* TLP2del1 for most carbon sources in the bean phyllosphere, and hence, *P. syringae* exhibited only a low level of coexistence with *P. fluorescens*. The relatively low level of coexistence of *P. fluorescens* with *P. syringae* in the bean phyllosphere, in contrast to the higher level of coexistence observed in a previous study with potatoes (56), suggests that the carbon sources utilized by *P. fluorescens*, but not by *P. syringae*, were more abundant in the potato phyllosphere than the bean phyllosphere. The *P. agglomerans* strains tested both exhibited variable levels of coexistence with *P. syringae* TLP2del1. The in vitro carbon source utilization data suggest that *P. syringae* competed with *P. agglomerans* 299R for most amino acids and organic acids but only certain carbohydrates. These data are supported by the nutrient amendment experiments, which indicated that most of the same amino acids alleviated the resource limitation of both the *P. agglomerans* and the *P. syringae* populations. The niche differentiation of *P. agglomerans* with respect to *P. syringae* occurred primarily in the utilization of carbohydrates; hence, the variable level of coexistence of *P. agglomerans* 299R with respect to *P. syringae* may reflect the variability in abundance of these carbohydrates in the bean phyllosphere. The variation in resource availability probably occurred because these replicate experiments were performed at different times of the year, since several environmental and physiological factors can affect the quality and quantity of exudates in the phyllosphere (13, 21, 25, 44, 51–53). *S. maltophilia* and *P. syringae* probably competed for few of the same amino acids or carbohydrates in the bean phyllosphere and hence exhibited a high level of coexistence. Presumably one or more of the carbon sources utilized by *S. maltophilia* BP1 were relatively abundant in the phyllosphere, because *S. maltophilia* BP1 achieved a population size sevenfold larger than that of *P. syringae* TLP2del1 when inoculated alone. *S. maltophilia* was able to catabolize several polymeric compounds in vitro, including starch and polygalacturonic acid, that *P. syringae* TLP2del1 was unable to catabolize. *M. organophilum* and *P. syringae* also probably competed for few of the same amino acids or carbohydrates in the phyllosphere and hence exhibited a high level of coexistence. The population size achieved by *M. organophilum* when inoculated alone was relatively small, probably reflecting the low abundance of carbon sources utilized by *M. organophilum* in the bean phyllosphere. *M. organophilum* strains are present in the phyllospheres of many plant species (14), and they may coexist with other epiphytic species in the phyllosphere by their unique ability to catabolize C_1 carbon sources such as methanol (14).

The relative population sizes of the competing strains in the replacement series were probably determined by several factors, including the abundance of the carbon sources that were utilized by both strains, the relative competitive ability of the strains for those carbon sources, and the abundance of the carbon sources that were utilized uniquely by each strain. The ability of a species or strain to catabolize a source of carbon not available to the majority of competing colonists may be one trait involved in epiphytic fitness (6, 38) or phyllosphere competence. This could be achieved either through catabolic versatility and the ability to utilize a diverse array of carbon sources which may be present only in low concentrations or through catabolic specialization and the ability to utilize an abundant carbon source which few other strains are able to utilize. Studies are currently under way in this laboratory to assess the contribution of the ability to utilize a unique carbon source upon epiphytic colonization and coexistence in epiphytic bacteria.

ACKNOWLEDGMENTS

This research was funded by a grant from the National Science Foundation (FD91-06782).

We acknowledge B. Larget for developing the statistical method used in these studies; V. J. Elliot and J. McInroy for strain identification; and G. A. Beattie, E. A. Clarke, N. Kokalis-Burelle, and W. Mahaffee for critical review of the manuscript.

REFERENCES

1. **Adee, S. R., W. F. Pfender, and D. C. Hartnett.** 1990. Competition between *Pyrenophora tritici-repentis* and *Septoria nodorum* in the wheat leaf as measured with de Wit replacement series. Phytopathology **80:**1177–1182.
2. **Andrews, J. H.** 1992. Biological control in the phyllosphere. Annu. Rev. Phytopathol. **30:**603–635.
3. **Andrews, J. H., C. M. Kenerley, and E. V. Nordheim.** 1980. Positional variation in phylloplane microbial populations within an apple tree canopy. Microb. Ecol. **6:**71–84.
4. **Austin, B., and M. Goodfellow.** 1978. Numerical taxonomy of phylloplane bacteria isolated from *Lolium perenne*. J. Gen. Microbiol. **104:**139–155.
5. **Bashi, E., and N. J. Fokkema.** 1977. Environmental factors limiting growth of *Sporobolomyces roseus*, an antagonist of *Cochliobolus sativus* on wheat leaves. Trans. Br. Mycol. Soc. **68:**17–25.
6. **Beattie, G. A., and S. E. Lindow.** Epiphytic fitness of phytopathogenic bacteria: physiological adaptations for growth and survival. Curr. Top. Microbiol. Immunol., in press.
7. **Blakeman, J. P.** 1985. Ecological succession of leaf surface microorganisms in relation to biological control, p. 6–30. *In* C. E. Windels and S. E. Lindow (ed.), Biological control on the phylloplane. American Phytopathological Society, St. Paul, Minn.
8. **Blakeman, J. P.** 1992. Fungal interactions on plant surfaces, p. 853–867. *In* G. C. Carroll and D. T. Wicklow (ed.), The fungal community: its organization and role in the ecosystem. Marcel Dekker, Inc., New York.
9. **Blakeman, J. P., and I. D. S. Brodie.** 1977. Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetroot leaves. Physiol. Plant Pathol. **10:**29–42.
10. **Brodie, I. D. S., and J. P. Blakeman.** 1975. Competition for carbon compounds by a leaf surface bacterium and conidia of *Botrytis cinerea*. Physiol. Plant Pathol. **6:**125–135.
11. **Clark, E., S. E. Lindow, and M. Wilson.** 1992. Chromosomal insertions of *xyl*E in *Pseudomonas syringae* as identifiable markers for ecological studies. Phytopathology **82:**1179. (Abstract.)
12. **Cody, Y. S., D. C. Gross, E. L. Proebsting, Jr., and R. A. Spotts.** 1987. Suppression of ice nucleation-active *Pseudomonas syringae* by antagonistic bacteria in fruit tree orchards and evaluation of frost control. Phytopathology **77:**1036–1044.
13. **Collins, M. A.** 1976. Colonization of leaves by phylloplane saprophytes and their interactions in this environment, p. 401–418. *In* C. H. Dickinson and T. F. Preece (ed.), Microbiology of aerial plant surfaces. Academic Press Ltd., London.
14. **Corpe, W. A., and S. Rheem.** 1989. Ecology of the methylotrophic

bacteria on living leaf surfaces. FEMS Microbiol. Ecol. **62:**243–250.
15. **Danilewicz, K.** 1980. Studies on the epiphytic bacteria on *Populus alba* L. at the time of optimal and minimal cambial activity and infection by *Venturia*. Eur. J. For. Pathol. **10:**296–306.
16. **Dickinson, C. H., B. Austin, and M. Goodfellow.** 1975. Quantitative and qualitative studies of phylloplane bacteria from *Lolium perenne*. J. Gen. Microbiol. **91:**157–166.
17. **Dik, A. J., N. J. Fokkema, and J. A. van Pelt.** 1992. Influence of climatic and nutritional factors on yeast population dynamics in the phyllosphere of wheat. Microb. Ecol. **23:**41–52.
18. **Edwards, M. C., and J. P. Blakeman.** 1984. An autoradiographic method for determining nutrient competition between leaf epiphytes and plant pathogens. J. Microsc. **133:**205–212.
19. **Ercolani, G. L.** 1978. *Pseudomonas savastanoi* and other bacteria colonizing the surface of olive leaves in the field. J. Gen. Microbiol. **109:**245–257.
20. **Evans, D. R., J. Hill, T. A. Williams, and I. Rhodes.** 1989. Coexistence and the productivity of white clover-perennial ryegrass mixtures. Theor. Appl. Genet. **77:**65–70.
21. **Fiala, V., C. Glad, M. Martin, E. Jolivet, and S. Derridj.** 1990. Occurrence of soluble carbohydrates on the phylloplane of maize (*Zea mays* L.): variations in relation to leaf heterogeneity and position on the plant. New Phytol. **115:**609–615.
22. **Fokkema, N. J.** 1984. Competition for endogenous and exogenous nutrients between *Sporobolomyces roseus* and *Cochliobolus sativus*. Can. J. Bot. **62:**2463–2468.
23. **Fokkema, N. J., J. G. den Houter, J. C. Kosterman, and A. L. Nelis.** 1979. Manipulation of yeasts on field-grown wheat leaves and their effects on *Cochliobolus sativus* and *Septoria nodorum*. Trans. Br. Mycol. Soc. **72:**19–29.
24. **Fravel, D. R.** 1988. Role of antibiosis in the biological control of plant diseases. Annu. Rev. Phytopathol. **26:**75–91.
25. **Godfrey, B. E. S.** 1976. Leachates from aerial parts of plants and their relation to plant surface microbial populations, p. 433–439. *In* C. H. Dickinson and T. F. Preece (ed.), Microbiology of aerial plant surfaces. Academic Press Ltd., London.
26. **Harper, J. L.** 1977. The population biology of plants. Academic Press Ltd., London.
27. **Hirano, S. S., and C. D. Upper.** 1992. Bacterial community dynamics, p. 271–294. *In* J. H. Andrews and S. S. Hirano (ed.), Microbial ecology of leaves. Springer-Verlag, New York.
28. **Hurlbert, S. H.** 1978. The measurement of niche overlap and some relatives. Ecology **59:**67–77.
29. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. **44:**301–307.
30. **Kinkel, L. L.** 1992. Fungal community dynamics, p. 253–270. *In* J. H. Andrews and S. S. Hirano (ed.), Microbial ecology of leaves. Springer-Verlag, New York.
31. **Kinkel, L. L., J. H. Andrews, F. M. Berbee, and E. V. Nordheim.** 1987. Leaves as islands for microbes. Oecologia **71:**405–408.
32. **Kinkel, L. L., and S. E. Lindow.** 1993. Invasion and exclusion among coexisting *Pseudomonas syringae* strains on leaves. Appl. Environ. Microbiol. **59:**3447–3454.
33. **Kranz, J.** 1981. Hyperparasitism of biotrophic fungi, p. 327–352. *In* J. P. Blakeman (ed.), Microbial ecology of the phylloplane. Academic Press Ltd., London.
34. **Lawlor, L. P.** 1980. Overlap, similarity and competition coefficients. Ecology **61:**245–251.
35. **Lindemann, J., and C. D. Upper.** 1985. Aerial dispersal of epiphytic bacteria over bean plants. Appl. Environ. Microbiol. **50:**1229–1232.
36. **Lindow, S. E.** 1985. Ecology of *Pseudomonas syringae* relevant to the field use of Ice^- deletion mutants constructed *in vitro* for plant frost control, p. 23–35. *In* H. O. Halvorson, D. Pramer, and M. Rogul (ed.), Engineered organisms in the environment: scientific issues. American Society for Microbiology, Washington, D.C.
37. **Lindow, S. E.** 1987. Competitive exclusion of epiphytic bacteria by Ice^- *Pseudomonas syringae* mutants. Appl. Environ. Microbiol. **53:**2520–2527.
38. **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.
38a. **Lindow, S. E.** Unpublished data.
39. **Lindow, S. E., D. C. Arny, and C. D. Upper.** 1983. Biological control of frost injury: an isolate of *Erwinia herbicola* antagonistic to ice nucleation active bacteria. Phytopathology **73:**1097–1102.
40. **Loper, J. E., and S. E. Lindow.** 1993. Roles of competition and antibiosis in suppression of plant diseases by bacterial biological control agents, p. 144–155. *In* R. D. Lumsden and J. L. Vaughn (ed.), Pest management: biologically based technologies. ACS symposium series. American Chemical Society, Washington, D.C.
41. **Ludwig, J. A., and J. F. Reynolds.** 1988. Statistical ecology: a primer on methods and computing, p. 111–124. John Wiley & Sons, Inc., New York.
42. **McBride, R. P., and A. J. Hayes.** 1979. Interactions of the leaf pathogen *Meria laricis*, with nutrients and microorganisms of the larch phylloplane. Nova Hedwigia **31:**507–517.
43. **Miller, J. H.** 1972. Experiments in bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Morgan, J. V., and H. B. Tukey, Jr.** 1964. Characterization of leachate from plant foliage. Plant Physiol. **39:**590–593.
45. **Morris, C. E.** 1985. Diversity of epiphytic bacteria on snap bean leaflets based on nutrient utilization abilities: biological and statistical considerations. Ph.D thesis. University of Wisconsin, Madison.
46. **Morris, C. E., and D. I. Rouse.** 1985. Role of nutrients in regulating epiphytic bacterial populations, p. 63–82. *In* C. E. Windels and S. E. Lindow (ed.), Biological control on the phylloplane. American Phytopathological Society, St. Paul, Minn.
47. **Niemela, J.** 1993. Interspecific competition in ground beetle assemblages (Carabidae): what have we learned? Oikos **66:**325–335.
48. **O'Brien, R. D., and S. E. Lindow.** 1989. Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. Phytopathology **79:**619–627.
49. **Pielou, E. C.** 1972. Niche width and overlap: a method for measuring them. Ecology **53:**687–692.
50. **Simberloff, D., and T. Dayan.** 1991. The guild concept and the structure of ecological communities. Annu. Rev. Ecol. Syst. **22:**115–143.
51. **Tukey, H. B.** 1966. The leaching of metabolites from above-ground plant parts and its implications. Bull. Torrey Bot. Club **93:**385–401.
52. **Tukey, H. B.** 1970. The leaching of substances from plants. Annu. Rev. Plant Physiol. **21:**305–324.
53. **Tukey, H. B.** 1971. Leaching of substances from plants, p. 67–80. *In* T. F. Preece and C. H. Dickinson (ed.), Ecology of leaf surface micro-organisms. Academic Press Ltd., London.
54. **Van Outryve, M. F., F. Gossele, and J. Swings.** 1989. The bacterial microflora of whitloof chicory (*Cichorium intybus* L. var. *foliosum* Hegi) leaves. Microb. Ecol. **18:**175–186.
55. **Wilson, M., and S. E. Lindow.** 1993. Interactions between the biological control agent *Pseudomonas fluorescens* strain A506 and *Erwinia amylovora* in pear blossoms. Phytopathology **83:**117–123.
56. **Wilson, M., and S. E. Lindow.** 1994. Ecological similarity and coexistence of epiphytic ice-nucleating (Ice^+) *Pseudomonas syringae* strains and a non-ice-nucleating (Ice^-) biological control agent. Appl. Environ. Microbiol. **60:**3128–3137.
57. **Wilson, M., and S. E. Lindow.** The significance of the ecological niche concept in biological control of epiphytic bacterial pests and pathogens. Mol. Ecol., in press.