APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1990, p. 2840–2846
0099-2240/90/092840-07$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 56, No. 9

Competition of Octopine-Catabolizing *Pseudomonas* spp. and Octopine-Type *Agrobacterium tumefaciens* for Octopine in Chemostats

COLIN R. BELL,[1]* NANCY E. CUMMINGS,[1] MARILYN L. CANFIELD,[2] AND LARRY W. MOORE[2]

Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada B0P 1X0,[1] and Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331[2]

Received 19 January 1990/Accepted 10 July 1990

The ability of two octopine-catabolizing *Pseudomonas* spp. and two virulent octopine-type *Agrobacterium tumefaciens* to compete for substrates has been examined in chemostats. In dual cultures with octopine or glutamate as the limiting carbon or nitrogen source, *Pseudomonas fluorescens* B99A and E175D always dominated over *A. tumefaciens* B6 or ATCC 15955. The growth dynamics of each strain in pure culture indicated that some form of antagonism was occurring in dual culture to permit the predominance of the pseudomonads under certain conditions. Although both pseudomonads fluoresce, pyoverdine was not responsible for the observed inhibition. An unidentified antibiotic secreted by both pseudomonads is believed to be responsible. *A. tumefaciens* B6 grew synergistically in the presence of *P. fluorescens* B99A with octopine as the limiting nitrogen source. This behavior of *Agrobacterium* strain B6 may help overcome its grossly inefficient use of octopine as previously reported. The ability of these two pseudomonads to outcompete the agrobacteria under all conditions tested raises the possibility that under field conditions, infectious agrobacteria may be succeeded by opine-catabolizing pseudomonads around crown gall tumors and in the rhizosphere.

The bacteria isolated from crown galls of a variety of plants invariably comprise more than just one type of virulent *Agrobacterium tumefaciens*. Representatives from biovar 1 and biovar 2 are often found together (1, 11, 17, 26, 28). If isolations are performed from grapevines, then all three biovars are possible (13, 34, 45). Frequently, isolates are reported with biovar-intermediate characteristics (8, 42, 43). Furthermore, many of the *Agrobacterium* isolates are nontumorigenic (9, 27, 38, 39), or if tumorigenic, they have widely different host specificities (3).

Data demonstrating the complexity of the crown gall community have recently increased to encompass other genera, principally pseudomonads (5, 36; M. L. Canfield, J. Boe, and L. W. Moore, Phytopathology **74**:1136, 1984) but also coryneforms (46). As the number and types of bacteria known to exploit opines in the tumorosphere or rhizosphere increase, so too do the number of questions about possible symbiotic relationships among the community members.

The opine concept (37, 44) has long stressed the role of opines as a carbon or nitrogen source for the inciting *Agrobacterium* sp. Competition between opine utilizers for limiting opine is therefore one obvious interaction. Because the majority of opine-catabolizing bacteria isolated to date are *Pseudomonas* spp., there is also an interest in the action of the many inhibitory agents secreted by pseudomonads (30) affecting the competition. Thus, antibiotics and the iron-transporting ability of the fluorescent pigments (32) could lead to antagonism against neighboring agrobacteria. The two octopine (O)-catabolizing *Pseudomonas* spp. in this study are both able to fluoresce. Their growth dynamics in pure culture have been previously reported (7), so it was possible in this study to distinguish pure competition from antagonism in dual cultures with virulent O-type *A. tumefaciens*.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. The two virulent biovar 1 strains of *A. tumefaciens* used in this study were B6 and ATCC 15955. The O-catabolizing pseudomonads were *Pseudomonas fluorescens* B99A and E175D. All four strains were from the culture collection of L. W. Moore.

Bacterial growth parameters and pyoverdine production. The maximum specific growth rate (μ_{max}) and K_s values for each strain were determined in pure chemostat cultures as reported previously (6, 7). Four different media were used. Mannitol glutamate (MG) and mannitol octopine (MO) media were nitrogen limited by the amino acids with a nitrogen concentration of 25 mg/liter. Glutamate (G) and O media were carbon limited with the amino acids as the sole source of carbon and nitrogen at 200 mg of C per liter. The curves relating growth rate (μ) to substrate concentration (S) were drawn from the Monod equation (33): $\mu = \mu_{max} S/(K_S + S)$.

Pyoverdine production in pure chemostat cultures was determined from steady-state samples which were all maintained at pH 7. Samples (2 to 3 ml) of culture were centrifuged at 11,500 × g. The A_{400} of the supernatant was measured in a 1-cm cuvette.

Competition studies. The theoretical basis for the competitions of the pseudomonads and the agrobacteria for a single growth-limiting substrate was based on the analysis by Powell (35) and follows the Monod equations:

$$\frac{dS}{dt} = (S_R - S)D - \frac{\mu_{max\,a}\, x_a S}{Y_a(K_a + S)} - \frac{\mu_{max\,b}\, x_b S}{Y_b(K_b + S)} \quad (1)$$

$$\frac{dx_a}{dt} = \frac{\mu_{max\,a}\, x_a S}{K_a + S} - Dx_a \quad (2)$$

$$\frac{dx_b}{dt} = \frac{\mu_{max\,b}\, x_b S}{K_b + S} - Dx_b \quad (3)$$

where S_R is the substrate concentration in the chemostat

reservoir (in milligrams per liter), S is the substrate concentration in the chemostat (in milligrams per liter), D is the dilution rate (hour^{-1}), $\mu_{max\ i}$ is the maximum specific growth rate of strain i (hour^{-1}), x_i is the concentration of strain i in the chemostat (in milligrams per liter), K_i is the substrate affinity (K_S) of strain i (in milligrams per liter), and Y_i is the yield coefficient of strain i.

In simple competition, with no interactions such as predation or antagonism, the strain with the Monod curve that can produce the highest growth rate on a given amount of substrate will dominate and displace all other strains from the chemostat (20, 29). Further analysis of dual cultures has indicated that the substrate concentration in the chemostat after inoculation decays exponentially for 6 to 10 h to reach a quasi-steady-state value approximately equal to the value it would attain if the more efficient strain were present in the chemostat by itself (18–20). At this point, the ratio of x_a to x_b can be represented by the following equation:

$$\frac{d \ln (x_a/x_b)}{dt} = \frac{\mu_{max\ a} S}{K_a + S} - \frac{\mu_{max\ b} S}{K_b + S} \quad (4)$$

Because all quantities on the right side of equation 4 are constants, a plot of ln (x_a/x_b) against time should produce a straight line. The slope of this line provides a convenient measure of the relative competitive ability of the two strains. The predicted outcome for all competitions was computed by the above equations and modeled with the Advanced Computer Simulation Language (Mitchell and Gauthier Associates, Concord, Mass.).

Bacteria for competition experiments were first grown overnight in shake flasks of the appropriate medium (MG, MO, O, or G) at 30°C. A 10-ml sample was then transferred into 300 ml of fresh medium and grown for 16 h at 30°C. The cell concentration of the broth was measured by acridine orange direct counts (23) and also with a Hawksley standard counting chamber (Hawksley, Lancing, Sussex, United Kingdom). Chemostats were filled with medium to within about 200 ml of the overflow. The required volumes of broth inoculum were added to bring the initial cell concentration of each strain to 5.0×10^8/ml. Fresh medium was rapidly added to bring the level up to the overflow, and the pump was switched on to give the desired dilution rate. The pseudomonads were distinguished from agrobacteria in chemostat samples by their different colony morphologies on MG agar plates, including fluorescence, and also by their resistance to the antibiotic triclosan (Irgasan) in Difco Pseudomonas Isolation Agar. Competitions were performed at 30°C, pH 7.

RESULTS

Simple competition for limiting substrate and agreement with simulations. Competition between *Pseudomonas* strain B99A and *Agrobacterium* strain ATCC 15955 in MG chemostats at a dilution rate of 0.2 h^{-1} (Fig. 1) produced excellent agreement between the observed data and that predicted from the Monod equations. The simple regression line (Fig. 1B) is a good fit ($r^2 = 0.947$), with an insignificant difference between the slope of this line (0.069) and that of the predicted line (0.083). The plate counts of each strain followed the predictions (Fig. 1C). The repeat of this experiment at a dilution rate of 0.15 h^{-1} (graphs not shown) produced equally good agreement between the observed line [$y = 0.58 + 0.057x$ ($r^2 = 0.098$)] and the expected line [$y = 1.29 + 0.083x$ ($r^2 = 1.0$)].

When *Pseudomonas* strain B99A and *Agrobacterium*

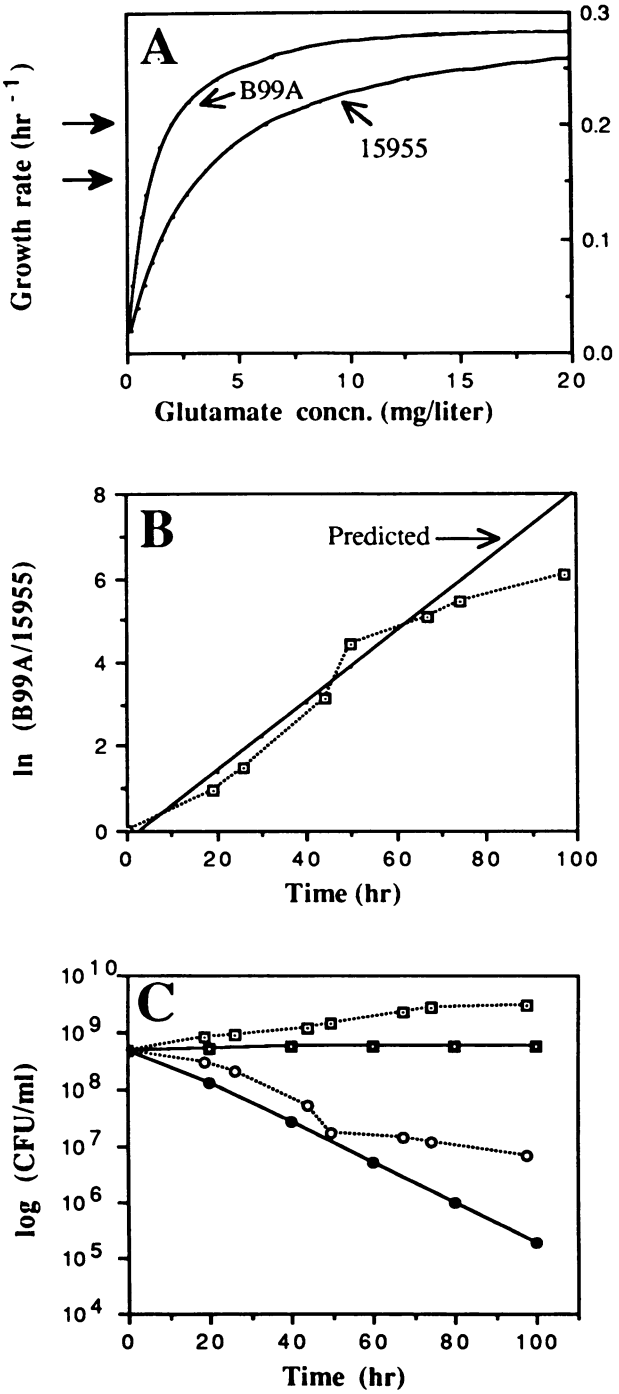

FIG. 1. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain ATCC 15955 on MG medium. (A) Monod growth curves. Arrows on the left show the dilution rates tested in chemostat experiments. (B) Outcome of the dual-culture experiment at 0.2 h^{-1}. The regression line for the predicted outcome is $y = -0.26 + 0.083x$ ($r^2 = 1.0$) and for the observed outcome is $y = 0.062 + 0.069x$ ($r^2 = 0.947$). (C) Plate counts at 0.2 h^{-1} Symbols: ⊡, number of *Pseudomonas* strain B99A CFU observed; ■, number of *Pseudomonas* strain B99A CFU predicted; ○, number of *Agrobacterium* strain ATCC 15955 CFU observed; ●, number of *Agrobacterium* strain ATCC 15955 CFU predicted.

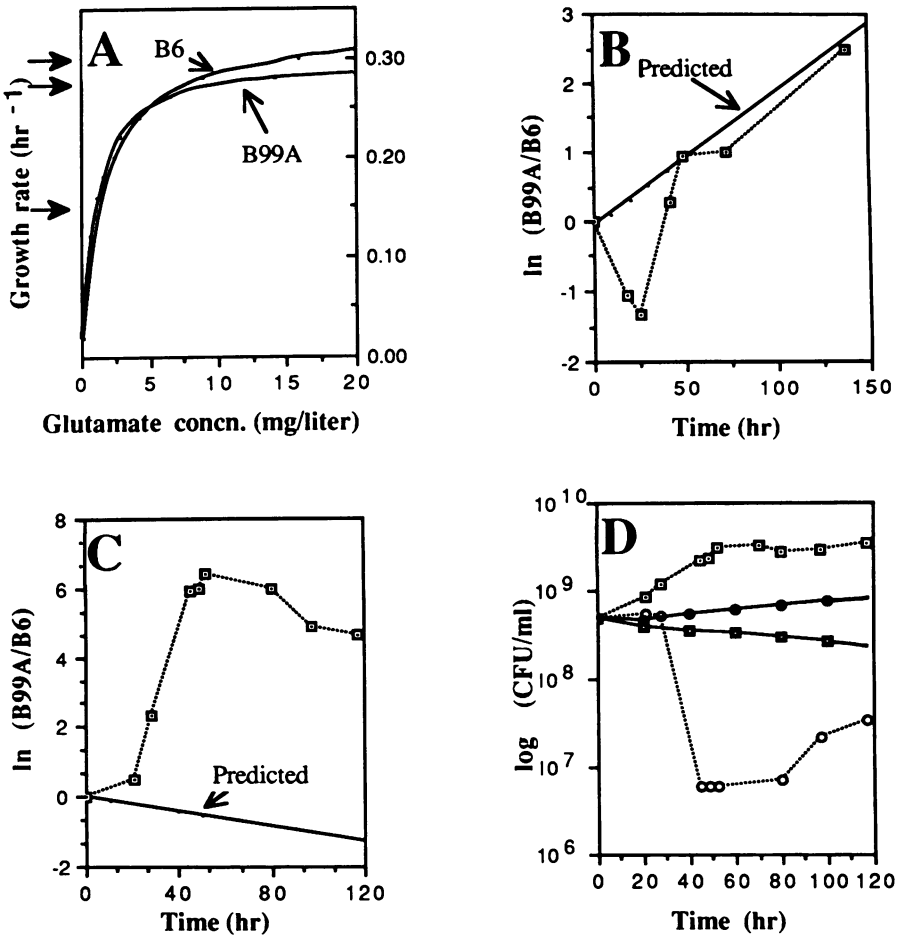

FIG. 2. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain B6 on MG medium. (A) Monod growth curves. Arrows on the left indicate the dilution rates tested. (B) Outcome of dual-culture experiment at 0.15 h^{-1}. The regression line for the predicted outcome is $y = -0.13 + 0.021x$ ($r^2 = 1.0$) and for the observed outcome is $y = -0.88 + 0.025x$ ($r^2 = 0.74$). (C) Outcome of the dual culture at 0.275 h^{-1}. The regression line for the predicted outcome is $y = 0.033 - 0.011x$ ($r^2 = 0.996$) and for the observed outcome is $y = 1.78 + 0.042x$ ($r^2 = 0.402$). (D) Plate counts at 0.275 h^{-1} Symbols: ⊡, number of *Pseudomonas* strain B99A CFU observed; ■, number of *Pseudomonas* strain B99A CFU predicted; ○, number of *Agrobacterium* strain B6 CFU observed; ●, number of *Agrobacterium* strain B6 CFU predicted.

strain B6 were run in dual cultures with MG medium at 0.15/h (Fig. 2A and B), only minor departures from predictions were observed. In the three cases described above, there was no persistent decrease in the numbers of agrobacteria below those predicted by the simulations.

Antagonism and the effect of dilution rate. When competitions between *Pseudomonas* strain B99A and *Agrobacterium* strain B6 were performed at higher dilution rates of 0.275 h^{-1} (Fig. 2C and D) and 0.30 h^{-1} (data not shown), drastic departures from the model occurred, suggesting some form of antagonism against *Agrobacterium* strain B6. *Agrobacterium* strain B6 should have dominated (Fig. 2A), according to simple competition theory. *Agrobacterium* strain B6 was severely inhibited and dropped below predicted numbers after about 30 h in the chemostat (Fig. 2D). *Pseudomonas* strain B99A had a μ_{max} in this medium of 0.30 h^{-1}. Thus, antagonism towards *Agrobacterium* strain B6 occurs at dilution rates ≥92% μ_{max} but not at 50% μ_{max} (i.e., 0.15 h^{-1}).

This antagonistic action is not restricted to *Pseudomonas* strain B99A. *Pseudomonas* strain E175D was also capable of inhibiting the growth of *Agrobacterium* strain B6 on the same MG medium at 0.2 h^{-1} (65% μ_{max}; data not shown). The number of CFU of *Agrobacterium* strain B6 fell below predictions after 45 h of chemostat culture.

Effect of substrate and pyoverdine on competitions. Under carbon limitation in G medium, *Pseudomonas* strain B99A displayed antagonism toward *Agrobacterium* strain B6 (Fig. 3). At a dilution rate of 0.20 h^{-1} (77% μ_{max}), inhibition was evident after 25 h (Fig. 3B); at 0.25 h^{-1} (96% μ_{max}), inhibition occurred after 20 h (data not shown).

With O as the limiting nitrogen substrate (MO medium, Fig. 4), antagonism against *Agrobacterium* strain ATCC 15955 caused a drop in numbers below predictions after 30 h. The dilution rate was 0.15 h^{-1} (71% μ_{max}). *Pseudomonas* strain E175D was also capable of inhibiting *Agrobacterium* strain ATCC 15955 when growing on O as the nitrogen source (Fig. 5). The dilution rate of 0.20 h^{-1} represents 95% μ_{max}. The predicted outcome was not linear because of the proximity of this dilution rate to the crossover point of the two curves (Fig. 5A).

When O was the limiting carbon source (O medium, Fig. 6) at a dilution rate of 0.10 h^{-1} (48% μ_{max}), inhibition of *Agrobacterium* strain ATCC 15955 was observed after 50 h in the chemostat. Pure culture studies of both *Pseudomonas* strain B99A and *Pseudomonas* strain E175D revealed that

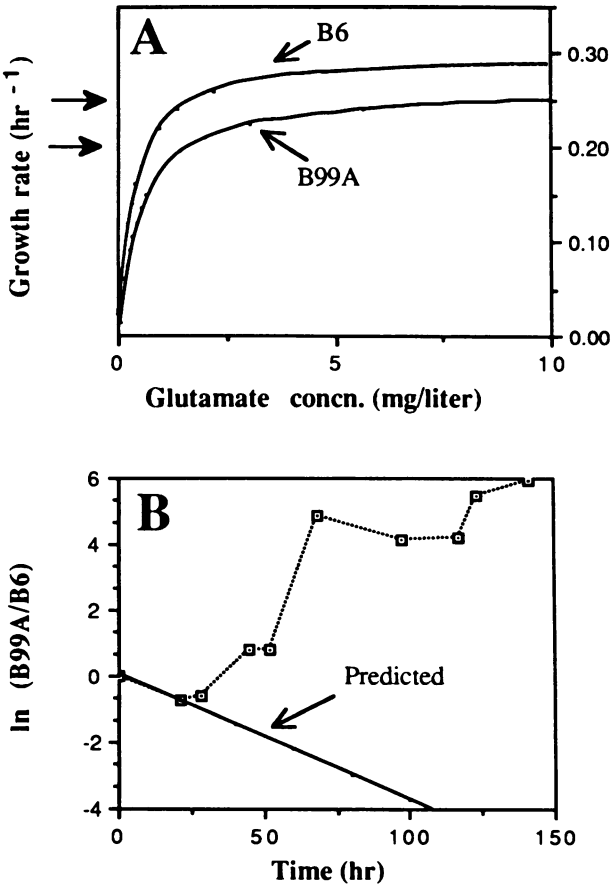

FIG. 3. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain B6 on G medium. (A) Monod growth curves. (B) Arrows on the left indicate the dilution rates tested. (B) Outcome of dual culture at 0.20 h^{-1}. The regression line for the predicted outcome is $y = 0.001 - 0.04x$ ($r^2 = 1.0$) and for the observed outcome is $y = -1.03 + 0.051x$ ($r^2 = 0.842$).

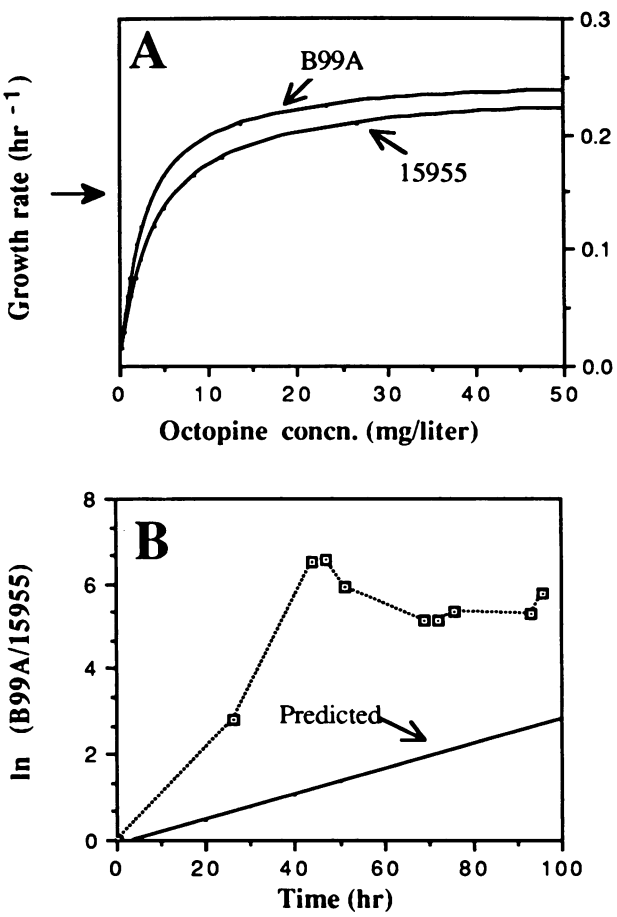

FIG. 4. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain ATCC 15955 on MO medium. (A) Monod growth curves. The arrow on the left indicates the dilution rate tested. (B) Outcome of dual culture at 0.15 h^{-1}. The regression line for the predicted outcome is $y = -0.08 + 0.029x$ ($r^2 = 1.0$) and for the observed outcome is $y = 2.25 + 0.045x$ ($r^2 = 0.454$).

in O medium, both pseudomonads produced negligible amounts of pyoverdine (Table 1). The lack of pyoverdine in O medium with dual cultures was apparent to the naked eye. The inhibitory agent under these conditions could not have been pyoverdine with the classical absorption maximum of 400 nm.

Susceptibility of the agrobacterial strains to antagonism. The highest concentrations of pyoverdine were consistently produced by *Pseudomonas* strain B99A under nitrogen limitation on MO medium (Table 1). Although *Agrobacterium* strain ATCC 15955 was significantly inhibited in this medium at 0.15 h^{-1} (Fig. 4), an identical run with *Pseudomonas* strain B99A and *Agrobacterium* strain B6 produced no inhibition of the *Agrobacterium* sp. (Fig. 7). *Pseudomonas* strain B99A was gradually dominating the dual culture but at a rate far slower than that predicted (Fig. 7B). Inspection of the plate counts (Fig. 7C) revealed that this was because *Agrobacterium* strain B6 was performing much better than was predicted. *Agrobacterium* strain B6 was not only resistant to any antibiotic-type effect from *Pseudomonas* strain B99A but was also growing synergistically in the presence of the pseudomonad.

DISCUSSION

In all dual cultures, the pseudomonads E175D and B99A were always able to dominate the culture. The medium, the dilution rate, and the agrobacterial strain did not alter this outcome. Further analysis of the bacterial populations in the chemostats indicated that at least three categories of population interaction accompanied the pseudomonad predominance. There was evidence of competition, antagonism, and commensalism (4, 21, 40).

Antagonism was the prevalent interaction, occurring in all four media and with both agrobacteria and both pseudomonads. Approximately 50 antibiotic substances have been characterized to date in pseudomonads (30). In fluorescent *Pseudomonas* spp., the water-soluble yellow-green pigment pyoverdine has received a great deal of attention because of its high complexing ability with Fe(III) (24, 25). This siderophore has been reported to be inhibitory to some bacteria (14, 31). Trace elements were added to all chemostat media used in these experiments to give a total iron concentration, by the phenanthroline assay (2), of 0.58 mg/liter.

Although additions of key nutrients to steady-state chemostats confirmed that either carbon or nitrogen was the limiting element, pyoverdine was initially suspected as the antagonistic agent. However, the inhibition encountered under carbon limitation on O medium, when pyoverdine was not produced, suggested that some other antibiotic was responsible. Furthermore, the lack of inhibition at low

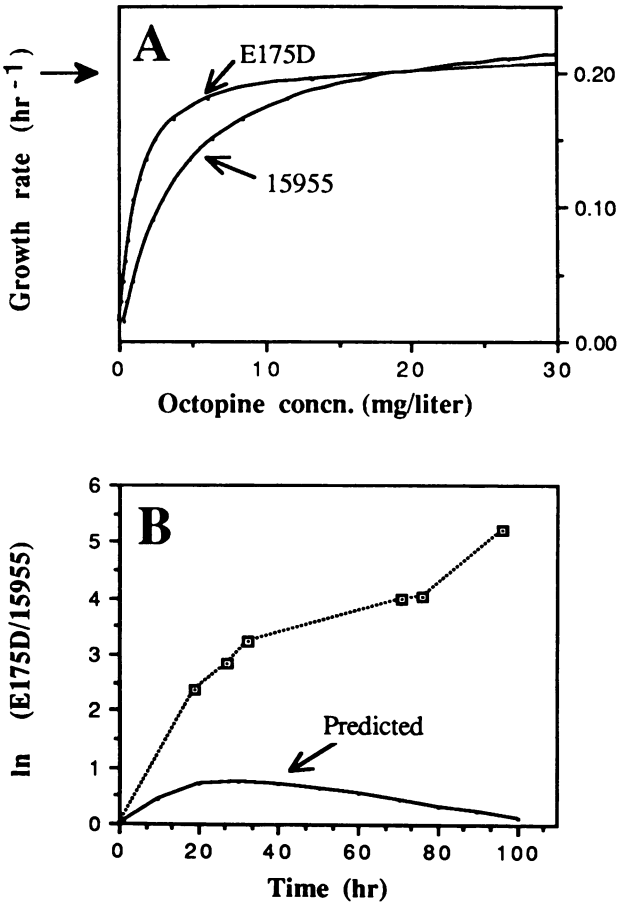

FIG. 5. Dual culture of *Pseudomonas* strain E175D and *Agrobacterium* strain ATCC 15955 on MO medium. (A) Monod growth curves. The arrowhead on the left indicates the dilution rate tested. (B) Outcome of dual culture at 0.20 h^{-1}. A regression line for the predicted outcome is not appropriate but for the observed outcome is $y = 1.11 + 0.043x$ ($r^2 = 0.85$).

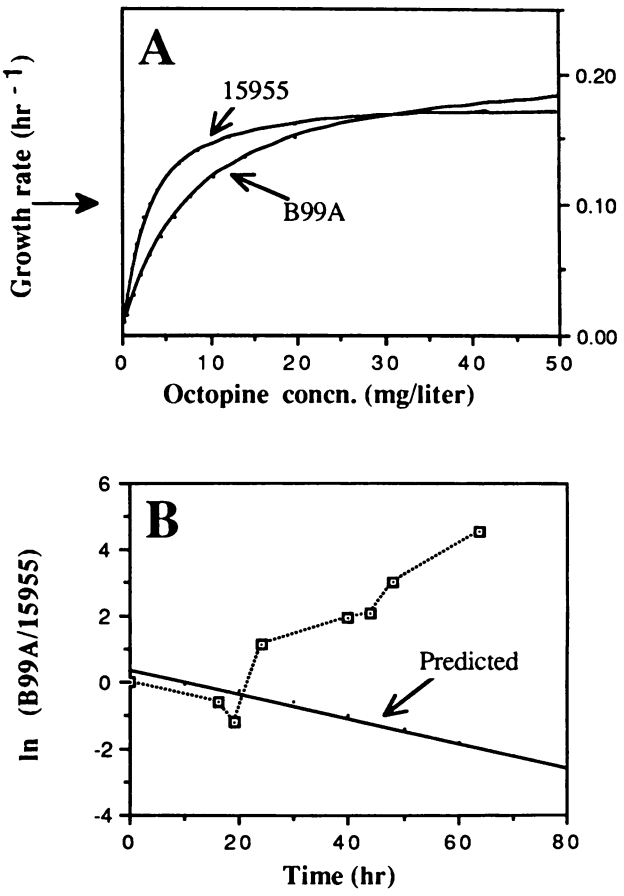

FIG. 6. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain ATCC 15955 on O medium. (A) Monod growth curves. The arrow on the left indicates the dilution rate tested. (B) Outcome of dual culture at 0.1 h^{-1}. The regression line for the predicted outcome is $y = 0.47 - 0.039x$ ($r^2 = 0.996$) and for the observed outcome is $y = -1.31 + 0.084x$ ($r^2 = 0.808$).

dilution rates on MG medium when pyoverdine was produced implied that under the iron conditions present in the chemostats, the pyoverdine was not involved in depressing the numbers of agrobacteria. We believe that both *Pseudomonas* strains B99A and E175D secrete an unidentified antibiotic inhibitory to the growth of *A. tumefaciens* ATCC 15955 and B6. The observed loss of antibiotic activity at dilution rates $\leq 60\%$ μ_{max} would then complement documented reports in the fermentation literature that the production of secondary metabolites is often reduced at low dilution rates or in early log phase (47).

The immunity of *Agrobacterium* strain B6 to antibiotic in MO medium may be attributable to the effect of the nitrogen source on the potency of the antibiotic. There was certainly a gross shift in the metabolism of all four bacterial strains when growing on MO compared with metabolism on the three other media. The volumes of acid and alkali necessary to maintain the steady-state pH of 7.0 were accurately monitored in all chemostats. Both G-based media (MG and G) and O medium made the culture alkaline, as might be expected with the ammonia release and possible secretion of basic amino acids from the catabolism of G and the reactions of the known arginine catabolic pathways after O cleavage (15, 16, 41). In contrast, MO medium made the culture acidic. Pyruvic acid (measured with the Sigma kit, assay number 726-UV) was detectable in the supernatant; presumably the carbon moiety of O can be dumped in nitrogen-limited conditions. Further investigations into this phenomenon which, it is hoped, will reveal the different susceptibilities of *Agrobacterium* strains ATCC 15955 and B6 to inhibition are under way.

The commensalism shown between *Pseudomonas* strain B99A and *Agrobacterium* strain B6 in MO medium may also prove to be mediated by metabolic byproducts. Nitrogenous products are necessary to account for the enhanced growth of *Agrobacterium* strain B6 in a nitrogen-limited chemostat;

TABLE 1. Pyoverdine concentrations in chemostat culture supernatant of pH 7[a]

Pseudomonad strain	Concn (A_{400} U) with[b]:			
	MG	G	MO	O
B99A	0.19	0.15	0.27	0.02
E175D	0.11	0.20	0.16	0.08

[a] Dilution rate, 0.15 h^{-1}.
[b] MG and MO, Nitrogen-limited media; G and O, carbon-limited media. Mean of three runs.

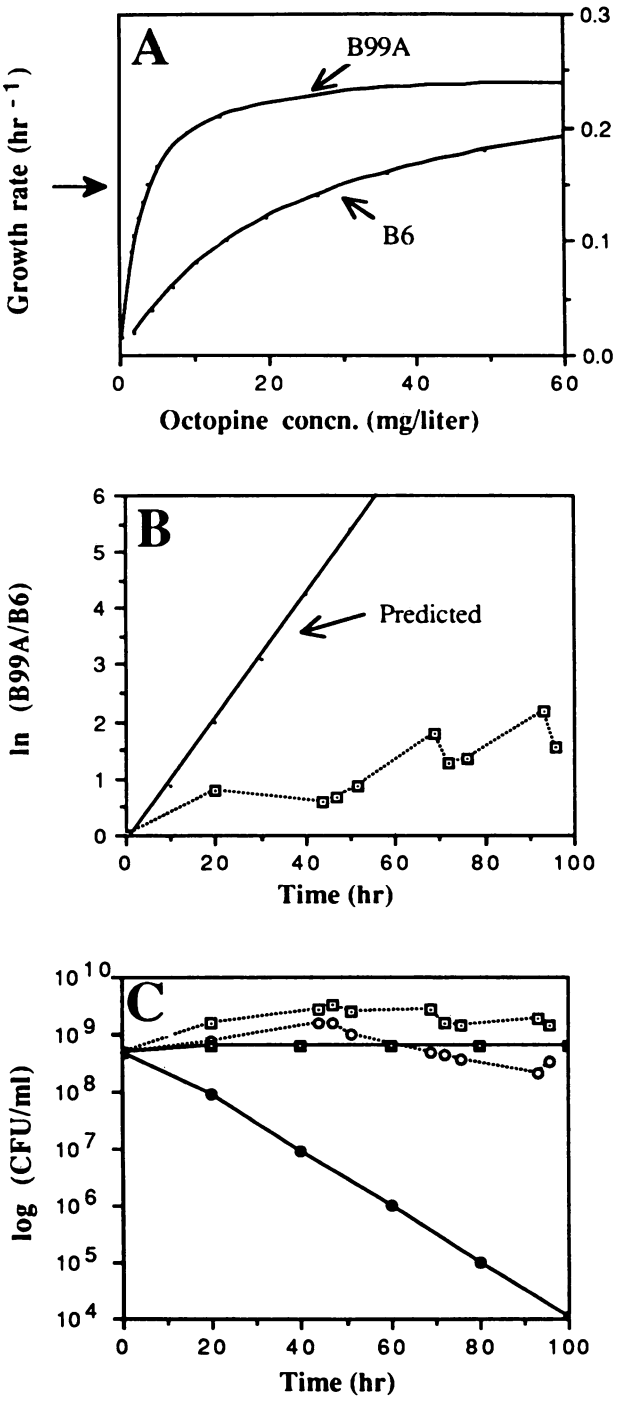

FIG. 7. Dual culture of *Pseudomonas* strain B99A and *Agrobacterium* strain B6 on MO medium. (A) Monod growth curves. The arrow on the left indicates the dilution rate tested. (B) Outcome of dual culture at 0.15 h^{-1}. The regression line for the predicted outcome is $y = -0.13 + 0.11x$ ($r^2 = 0.998$) and for the observed outcome is $y = 0.052 + 0.019x$ ($r^2 = 0.795$). (C) Plate counts at 0.15 h^{-1}. Symbols: ⊡, number of *Pseudomonas* strain B99A CFU observed; ■, number of *Pseudomonas* strain B99A CFU predicted; ○, number of *Agrobacterium* strain B6 CFU observed; ●, number of *Agrobacterium* strain B6 CFU predicted.

ammonia is a likely candidate. The gross inefficiency of O assimilation in *Agrobacterium* strain B6 would favor the utilization of other nitrogen supplies. *Pseudomonas* strain B99A secretes approximately 55 nmol of ammonia per mg of cell dry weight per h when grown in pure chemostat culture (assay method of Harwood and Kuhn [22]). This, if combined with other nitrogenous byproducts, may be sufficient to sustain *Agrobacterium* strain B6. To our knowledge, the ammonia assimilation of *Agrobacterium* spp. has not been investigated. However, it is intriguing to note that in *Rhizobium* spp., the NADP-glutamate dehydrogenase pathway is undetectable and only the high-affinity glutamine synthetase-glutamate synthase pathway is operational (10). It is this latter pathway that would be advantageous with these low levels of ammonia (12).

It is interesting to consider whether the competitive superiority of these two pseudomonads for one limiting substrate under all chemostat conditions tested will extend to multisubstrate conditions in the field. The secretion of an antibiotic, together with the siderophore activity of pyoverdine in low-iron environments, can only enhance their competitiveness. Niches based on O, and probably other opines, will quickly be exploited by such pseudomonads. In plants, the opine niche may be initiated by virulent *A. tumefaciens*, but that may be displaced by a succession of other opine-catabolizing genera, particularly *Pseudomonas* spp.

ACKNOWLEDGMENT

This work was supported by operating grant A3190 from the Natural Sciences and Engineering Council of Canada.

LITERATURE CITED

1. **Alconero, R.** 1980. Crown gall of peaches from Maryland, South Carolina and Tennessee and problems with biological control. Plant Dis. **64**:835–838.
2. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
3. **Anderson, A. R., and L. W. Moore.** 1979. Host specificity in the genus *Agrobacterium*. Phytopathology **64**:320–323.
4. **Bazin, M. J.** 1981. Mixed culture kinetics, p. 25–51. *In* M. E. Buskell and J. H. Slater (ed.), Mixed culture fermentations. Academic Press, Inc., New York.
5. **Beaulieu, J., L. J. Coulombe, R. L. Granger, B. Miki, C. Beauchamp, G. Rossignol, and P. Dion.** 1983. Characterization of opine-utilizing bacteria isololated from Quebec. Phytoprotection **64**:61–68.
6. **Bell, C. R.** 1990. Growth of *Agrobacterium tumefaciens* under octopine limitation in chemostats. Appl. Environ. Microbiol. **56**:1775–1781.
7. **Bell, C. R., L. W. Moore, and M. L. Canfield.** 1990. Growth of octopine-catabolizing *Pseudomonas* spp. under octopine limitation in chemostats and their potential to compete with *Agrobacterium tumefaciens*. Appl. Environ. Microbiol. **56**:2834–2839.
8. **Bouzar, H., and L. W. Moore.** 1987. Isolation of different *Agrobacterium* biovars from a natural oak savanna and tallgrass prairie. Appl. Environ. Microbiol. **53**:717–721.
9. **Bouzar, H., L. W. Moore, and N. W. Schaad.** 1983. Crown gall of pecan: a survey of *Agrobacterium* strains and potential for biological control in Georgia. Plant Dis. **67**:310–312.
10. **Bravo, A., and J. Mora.** 1988. Ammonium assimilation in *Rhizobium phaseoli* by the glutamine synthetase-glutamate synthase pathway. J. Bacteriol. **170**:980–984.
11. **Brisbane, P. G., and A. Kerr.** 1983. Selective media for three biovars of *Agrobacterium*. J. Appl. Bacteriol. **54**:425–431.
12. **Brown, C. M., D. S. MacDonald-Brown, and S. O. Stanley.** 1973. The mechanisms of nitrogen assimilation in pseudomonads. Antonie van Leeuwenhoek J. Microbiol. **39**:89–98.
13. **Burr, T. J., B. H. Katz, and A. L. Bishop.** 1987. Populations of

Agrobacterium in vineyard and non-vineyard soils and grape roots in vineyards and nurseries. Plant Dis. **71**:617–620.
14. **Buyer, J. S., and J. Leong.** 1986. Iron transport-mediated antagonism between plant growth-promoting and plant-deleterious *Pseudomonas* strains. J. Biol. Chem. **261**:791–794.
15. **Clarke, P. H., and N. Ornston.** 1975. Metabolic pathways and regulation II, p. 263–340. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, Inc., London.
16. **Dessaux, Y., A. Petit, J. Tempé, M. Demarez, C. Legrain, and J.-M. Wiame.** 1986. Arginine catabolism in *Agrobacterium* strains: role of the Ti plasmid. J. Bacteriol. **166**:44–50.
17. **du Plessis, H. J., H. J. J. van Vuuren, and M. J. Hattingh.** 1984. Biotypes and phenotypic groups of strains of *Agrobacterium* in South Africa. Phytopathology **74**:524–529.
18. **Dykhuizen, D., and D. Hartl.** 1981. Evolution of competitive ability in *Escherichia coli*. Evolution **35**:581–594.
19. **Dykhuizen, D., and D. L. Hartl.** 1980. Selective neutrality of 6PGD allozymes in *E. coli* and the effects of genetic background. Genetics **96**:801–817.
20. **Dykhuizen, D. E., and D. L. Hartl.** 1983. Selection in chemostats. Microbiol. Rev. **47**:150–168.
21. **Frederickson, A. G., and G. Stephanopoulos.** 1981. Microbial competition. Science **213**:972–979.
22. **Harwood, J. E., and A. L. Kuhn.** 1970. A colorimetric method for ammonia in natural waters. Water Res. **4**:805–811.
23. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
24. **Hohnadel, D., and J. M. Meyer.** 1986. Pyoverdine-facilitated iron uptake among fluorescent pseudomonads, p. 119–129. *In* T. R. Swinburne (ed.), Iron, siderophores and plant diseases. Plenum Publishing Corp., New York.
25. **Hubbard, J. P., G. E. Harman, and Y. Hadar.** 1983. Effect of soilborne *Pseudomonas* spp. on the biological control agent *Trichoderma hamatum* on pea seeds. Phytopathology **37**:655–659.
26. **Kerr, A.** 1969. Crown gall of stone fruit. I. Isolation of *Agrobacterium tumefaciens* and related species. Aust. J. Biol. Sci. **22**:111–116.
27. **Kerr, A.** 1974. Soil microbiological studies on *Agrobacterium radiobacter* and biological control of crown gall. Soil Sci. **118**:168–172.
28. **Kerr, A., and C. G. Panagopoulos.** 1977. Biotypes of *Agrobacterium rhizogenes* var. *tumefaciens* and their biological control. Phytopathol. Z. **90**:172–179.
29. **Kuenen, J. G., and W. Harder.** 1982. Microbial competition in continuous culture, p. 342–367. *In* R. G. Burns and J. H. Slater (ed.), Experimental microbial ecology. Blackwell Scientific Publications, Ltd., Oxford.
30. **Leisinger, T., and R. Margraff.** 1979. Secondary metabolites of the fluorescent pseudomonads. Microbiol. Rev. **43**:422–442.
31. **Leong, J.** 1986. Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. Annu. Rev. Phytopathol. **24**:187–209.
32. **Meyer, J. M., and M. A. Abdallah.** 1978. The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physiochemical properties. J. Gen. Microbiol. **107**:319–328.
33. **Monod, J.** 1942. Récherches sur la croissance des cultures bacteriénnes. Herman, Paris.
34. **Panagopoulos, C. G., and P. G. Psallidas.** 1973. Characteristics of Greek isolates of *Agrobacterium tumefaciens* (E. F. Smith & Townsend) Conn. J. Appl. Bacteriol. **36**:233–240.
35. **Powell, E. O.** 1958. Criteria for the growth of contaminants and mutants in continuous culture. J. Gen. Microbiol. **18**:259–268.
36. **Rossignol, G., and P. Dion.** 1985. Octopine, nopaline and octopinic acid utilization in *Pseudomonas*. Can. J. Microbiol. **31**:68–74.
37. **Schell, J., M. Van Montagu, M. De Beuckeleer, M. De Block, A. Depicker, M. DeWilde, G. Engler, C. Genetello, J. P. Hernalsteens, M. Holsters, J. Seurinck, B. Silva, F. Van Vliet, and R. Villaroel.** 1979. Interaction and DNA transfer between *Agrobacterium tumefaciens*, the Ti plasmid and the plant host. Proc. R. Soc. London Ser. B **204**:251–266.
38. **Schroth, M. N., J. P. Thompson, and D. C. Hildebrand.** 1965. Isolation of *Agrobacterium tumefaciens-A. radiobacter* group from soil. Phytopathology **55**:645–647.
39. **Schroth, M. N., A. R. Weinhold, A. H. McCain, D. C. Hildebrand, and N. Ross.** 1971. Biology and control of *Agrobacterium tumefaciens*. Hilgardia **40**:537–552.
40. **Slater, J. H., and A. T. Bull.** 1978. Interactions between microbial populations, p. 181–206. *In* A. T. Bull and P. M. Meadow (ed.), Companion to microbiology. Longman, London.
41. **Sokatch, J. R.** 1969. Bacterial physiology and metabolism. Academic Press, Inc., New York.
42. **Spiers, A. G.** 1979. Isolation and characterization of *Agrobacterium* species. N. Z. J. Agric. Res. **22**:631–636.
43. **Süle, S.** 1978. Biotypes of *Agrobacterium tumefaciens* in Hungary. J. Appl. Bacteriol. **44**:207–213.
44. **Tempé, J., and A. Petit.** 1983. La piste des opines, p. 14–32. *In* A. Puhler (ed.), Molecular genetics of the bacteria-plant interaction. Springer-Verlag, New York.
45. **Thomson, J. A.** 1987. The use of agrocin-producing bacteria in the biological control of crown gall, p. 213–228. *In* I. Chet (ed.), Innovative approaches to plant disease control. John Wiley & Sons, Inc., New York.
46. **Tremblay, G., R. Gagliardo, W. S. Chilton, and P. Dion.** 1987. Diversity among opine-utilizing bacteria: identification of coryneform isolates. Appl. Environ. Microbiol. **53**:1519–1524.
47. **Wang, D. I. C., C. L. Cooney, A. L. Demain, P. Dunnill, A. E. Humphrey, and M. D. Lilly.** 1979. Fermentation and enzyme technology. John Wiley & Sons, Inc., New York.