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Growth of *Agrobacterium tumefaciens* under Octopine Limitation in Chemostats

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***Agrobacterium tumefaciens* B6 and ATCC 15955 were grown under octopine or glutamate limitation in chemostats. Examination of the maximum specific growth rate (μ_{max}) and substrate affinity (K_S) for each strain indicated that strain B6 was highly inefficient in its use of octopine as either a nitrogen or carbon source compared with strain ATCC 15955. Examination of the yield coefficients showed that in both strains octopine was used more efficiently as a nitrogen source than as a carbon source. The data permitted predictions to be made concerning the outcome of competition for a single limiting substrate. Under octopine limitation, strain ATCC 15955 should dominate; under glutamate limitation, strain B6 should dominate. The results of an observed competition with glutamate as the limiting substrate confirmed the latter prediction, although B6 did dominate at a rate faster than was predicted from simple competition theory. B6 displayed higher growth rates and substrate affinities than ATCC 15955 on all concentrations of glutamate. The yield of B6 on octopine was also considerably higher. This latter attribute could provide an ecological advantage to B6 because of the importance of yield in the fate of competitions under multisubstrate regimens. These will be the most prevalent regimens in the area around the tumor (tumorosphere) or the rhizosphere. The increased performance on glutamate could provide an advantage in an opine-free environment when B6 is growing as a saprophyte.**

The opine concept (50, 51), or the genetic colonization theory (40), occupies a key position in the understanding of the bacteriology of crown gall disease. The concept states that the opines synthesized and secreted by the transformed plant tissue create an ecological niche favorable to the *Agrobacterium tumefaciens* which initiated the infection.

The correspondence between the opine synthesis genes on the T-DNA (31, 41) and the opine catabolism genes on other regions of the Ti plasmid (4, 29, 30) has led to significant discoveries in the field. Thus, predictions based on the theory were instrumental in the identification of agropine from null-type tumors (15, 17) and the discovery of agrocinopines (14) in agrocin-sensitive strains. The validity and robustness of the concept allowed it to be extended to describe the opines of *Agrobacterium rhizogenes* (35), and it may prove useful in elaborating the role of opine-like compounds in rhizobia (32).

The assumption that the opine niche belongs exclusively to the genus *Agrobacterium* has had to be modified recently to include other genera, principally pseudomonads and coryneforms, which can catabolize opines (2, 39, 53; M. L. Canfield, J. Boe, and L. W. Moore, Phytopathology **74**:1136, 1984). As the complexity of the bacterial community able to exploit the opine niche increases, basic physiological information on growth is required to appreciate the interactions of the community members. Tempé et al. (52), in describing the parasite's point of view of opine ecology, postulated that because opines permit the multiplication of the bacteria and also induce the conjugation of the Ti plasmid, selection could operate on the bacterium, the Ti plasmid, or the T-DNA. Accurate descriptions of the growth of agrobacteria on opines would help us to ascertain the importance of the first possibility.

All previous experimentation on the growth of agrobacteria has involved batch culture (4, 7, 16, 23–26, 29, 30, 34, 36, 49). The chemostat provides more precise control of the environment for the determination of key growth parameters, in addition to providing the ability to discriminate between the use of opines as a source of nitrogen or of carbon. This study investigates the growth of two virulent *A. tumefaciens* strains under octopine limitation in chemostats. The growth performances of the two strains under glutamate limitation, a major breakdown product of octopine catabolism (9, 13) and octopine limitation, are compared.

MATERIALS AND METHODS

Bacterial strains. The two agrobacterial strains used in this study were B6 and ATCC 15955. Both are virulent biovar 1 strains and were kindly supplied by L. W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis.

Batch culture conditions. A mannitol-glutamate medium (MG/B) of the following composition (grams per liter) was used: mannitol, 5.0; L-glutamic acid, 2.1; K_2HPO_4, 0.5; NaCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; and biotin stock solution (2 μg/ml), 1 ml/liter. All batch culture media were adjusted to pH 7.0 unless otherwise indicated.

Maximum and minimum temperatures for growth were determined by inoculating test tubes containing MG/B broth and incubating them in an aluminum temperature gradient block. Growth was tested at temperature increments of 1°C. Optimum temperatures were determined in 250-ml shake flasks in a controlled-environment shaker (Lab-Line Bioengineering Ltd., Melrose Park, Ill.) at 200 rpm at 2°C increments.

Maximum and minimum pHs for growth were determined in MG/B broth with double the amount of phosphate buffer. Medium in test tubes was adjusted to a pH of 4.0 to 12.0 in increments of 0.5 pH units. Incubations were performed at 30°C on a rotating test tube platform (Cole Parmer Instrument Co., Chicago, Ill.). The final pH of tubes in which

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growth occurred was always found to be 2 to 3 pH units closer to 7.0 than was the initial pH. In the determinations of pH optima, this modification of the pH was avoided by monitoring the growth rate in chemostats (see below for details) operated in batch mode. Both chemostats had pH controllers with acid and alkali addition pumps. The accuracy of pH control was ±0.1 pH unit. Growth curves were obtained at intervals of 0.25 pH unit.

The maximum specific growth rate (μ_{max}) was determined from growth curves obtained in shake flasks at 30°C, pH 7.0. In all of the experiments described above, growth was measured turbidimetrically at 600 nm (Spectronic 20; Bausch and Lomb, Rochester, N.Y.). All determinations were repeated at least three times.

Continuous culture conditions. Two aerobic benchtop chemostats with 500-ml working volumes were used, an LH 500 series fermentor (LH Fermentation Ltd., Slough, England) and a Pegasus Versatec fermentor (Pegasus Industrial Specialties Ltd., Agincourt, Ontario, Canada). Both vessels had temperature and pH control. Agitation was applied by magnetic stirrers at 500 rpm.

The nitrogen and carbon constituents of MG/B broth were modified for use in chemostats to produce four different media. The two nitrogen-limited media each contained 25 mg of N per liter and consisted of mannitol-glutamate (MG) (glutamate, 0.33 g/liter; mannitol, 1.58 g/liter) and mannitol-octopine (MO) [D-(+)-octopine (Sigma Chemical Co., St. Louis, Mo.), 0.11 g/liter; mannitol, 1.58 g/liter]. The two carbon-limited media each contained 200 mg of C per liter and consisted of glutamate (G medium) (glutamate, 0.624 g/liter; no mannitol) and octopine (O medium) [D-(+)-octopine, 0.45 g/liter; no mannitol]. All other salts were as in MG/B except that 1 ml of each of the following trace element solutions was added per liter. Solution A consisted of the following components (in grams per liter): $CaCl_2 \cdot 2H_2O$, 4.0; $MnCl_2 \cdot 4H_2O$, 4.0; $FeCl_3 \cdot 6H_2O$, 2.8. Solution B consisted of the following components: $CoCl_2 \cdot 6H_2O$, 0.2 g/liter; ammonium molybdate, 0.2 g/liter. Solution C consisted of the following components (in grams per liter): $ZnSO_4 \cdot 7H_2O$, 0.1; $SrCl_2 \cdot 6H_2O$, 0.2; $NiCl_2 \cdot 6H_2O$, 0.2; boric acid, 0.2; KI, 0.1. The limitation imposed by each medium was confirmed by adding carbon or nitrogen supplements to cultures and observing any changes in steady state biomass.

Inoculum for each chemostat run was obtained from overnight shake flask cultures of agrobacteria in the appropriate medium. Cells at an optical density at 600 nm of 0.1 (approximately 7.0×10^7 cells per ml) in 75 ml of medium were added to the chemostat vessel equilibrated to pH 7.0, 30°C. Growth proceeded under batch culture conditions for the first 10 to 12 h before the medium pumps were switched on. The dilution rate for all runs was 0.15 h^{-1}. Steady state samples were taken after five volume changes. Biomass and substrate determinations confirmed that steady state had been achieved. Three chemostat runs were performed for each agrobacterium under each growth regimen.

Growth and yield parameters. The maximum specific growth rate in the chemostat was estimated by increasing the dilution rate to approximately 25% above μ_{max} and monitoring washout kinetics (46). Substrate affinity (K_s) was calculated from a Lineweaver-Burk linearization of the Monod equation (28), $\mu = \mu_{max}\,[S/(K_S + S)]$, after determining steady state substrate concentrations from culture supernatant. A small steady state sample was centrifuged in a Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) for 2 min at 11,500 × *g*. In order to minimize substrate assimilation, centrifugation commenced within 15 s of taking the sample. Glutamate was assayed by using glutamate dehydrogenase, diaphorase, and tetrazolium salts (3). D-(+)-Octopine was assayed by the method of Johnson et al. (20). The yield was calculated from the steady state cell dry weight. Thirty-five milliliters of steady-state cell culture was pelleted at 5,000 × *g*. The cells were suspended in 10 ml of distilled water and dried at 90°C in a preweighed aluminum pan for 24 h. Simulations were run with the calculated values for K_S, μ_{max}, and yield by using Advanced Continuous Simulation Language (ACSL) (Mitchell and Gauthier Associates, Concord, Mass.). The agreement between observed and expected steady state values was excellent.

The productivity of cells is the product of the cell dry weight and the dilution rate. The number of cells per milliliter was determined by microscopy after staining with acridine orange (19). Cells were counted with a Leitz Laborlux 12 microscope fitted with a 50-W ultra-high-pressure mercury lamp. The excitation range was 390 to 490 nm (filter block H2) with a 515-nm suppression filter. The average cell size was estimated from the quotient of cell dry weight and epifluorescence cell counts.

Competition experiment and theory. Cultures of strains B6 and ATCC 15955 for the competition run were first grown overnight from a solid inoculum in MG/B in shake flasks at 30°C. Ten milliliters was then transferred to 300 ml of fresh MG/B and grown for 16 h at 30°C. The cell concentration in the broth was measured by acridine orange direct counts and also with a 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 and inocula were added to bring the initial cell concentration of each strain to 5.0×10^8/ml. Fresh medium was added to rapidly bring the level up to the overflow, and the pump was switched on at a dilution rate of 0.15 h^{-1}. Samples were taken twice each day for 5 days. ATCC 15955 was distinguishable from B6 by its different colony morphology on MG/B plates and also by its ability to grow on MG/B plus 100 μg of nalidixic acid per ml.

The behavior of two strains, a and b, competing for the same limiting substrate was first discussed by Powell (38) and follows the Monod equations:

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

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

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

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

In simple competition, with no interactions such as predation or antagonism, S 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 (10–12). At this point, the ratio of x_a to x_b can be represented by:

TABLE 1. Growth data for strains in continuous culture (dilution rate, 0.15 h^{-1})

Strain and growth medium	μ_{max} (h^{-1})	K_S (mg of glutamate/liter)	K_S (mg of octopine/liter)	K_S (mg of C/liter)	K_S (mg of N/liter)
B6					
MG	0.33	1.58			0.12
G	0.30	0.34		0.11	
MO	0.27		24.73		5.62
O	0.21		36.89	16.18	
ATCC 15955					
MG	0.30	3.16			0.24
G	0.25	1.08		0.34	
MO	0.24		3.82		0.87
O	0.18		2.45	1.08	

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

Because all quantities on the right hand 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 B6 and ATCC 15955 was determined by using these equations and iterated on ACSL.

RESULTS

Batch culture characteristics. The growth rate, together with the cardinal temperatures and pH values, of the two agrobacteria in batch culture suggested that the two strains were very similar. The two strains, when grown in MG/B medium in batch culture, exhibited the same minimum pH for growth (4.5), maximum pH for growth (11), and optimal pH for growth (7.25). In addition, they exhibited the same maximum temperature for growth (46°C) and optimum temperature for growth (30°C). The minimum temperature for growth for B6 was 6°C; that for ATCC 15955 was 5°C. The maximum specific growth rate at optimum pH and temperature was 0.29 h^{-1} for B6; that for ATCC 15955 was 0.32 h^{-1}. It was only in chemostat culture, with different media, that consistent distinctions arose.

Growth rates and K_S values in chemostats. An examination of the effect of glutamate versus that of octopine on the maximum growth rate (μ_{max}) of both B6 and ATCC 15955 (Table 1) showed that μ_{max} was reduced considerably with octopine. This occurred whether the amino acids were supplied in nitrogen- or carbon-limited media. When the rates were compared within glutamate-based media (MG and G medium) and within octopine-based media (MO and O medium), both substrates sustained faster growth rates when fed as the nitrogen-limiting nutrient.

The K_S data in Table 1 provide an estimate of the efficiency of substrate utilization and indicate that strains B6 and ATCC 15955 were comparable when growing on glutamate but that B6 produced high K_S values (24.73 and 36.89) when growing on octopine. This represents an inefficient use of octopine by B6 which can be seen more clearly when K_S values are converted to milligrams of carbon or nitrogen. Under nitrogen limitation, comparing MG with MO, there was a 50-fold decrease in efficiency (0.12 to 5.62), while carbon limitation produced a 150-fold decrease (0.11 to 16.18; G medium compared with O). ATCC 15955 suffered a less drastic decrease when growing on octopine compared with growth on glutamate, showing only a three- and four-fold reduction in growth for carbon and nitrogen limitation, respectively.

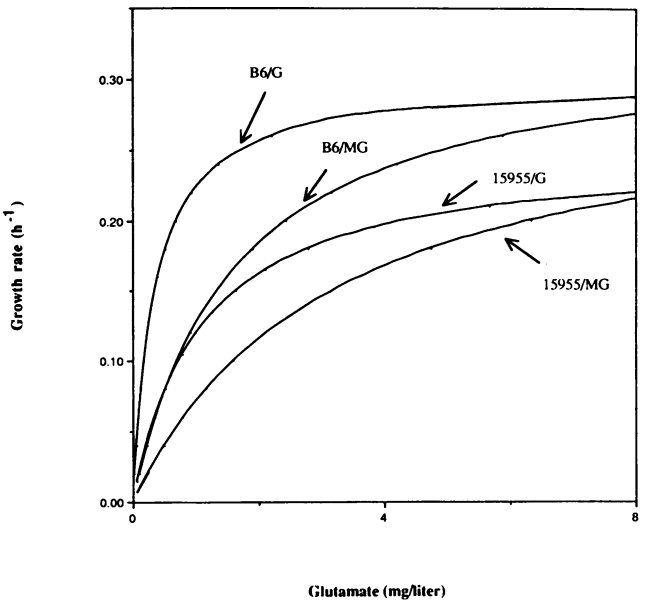

FIG. 1. Monod curves for B6 and ATCC 15955 with glutamate as the limiting nitrogen source (MG) or limiting carbon source (G).

The interplay of K_S and μ_{max} can best be appreciated when the plots for growth rate (μ) and substrate concentration (S) are drawn according to the Monod relationship: $\mu = \mu_{max}[S/(K_S + S)]$. Figures 1 to 4 have been drawn to show predictions at a low substrate concentration because such conditions are assumed to be of more ecological relevance. At these subsaturating concentrations of substrate, growth is controlled by the limiting nutrient, i.e., glutamate or octopine. Mannitol is added as a supplemental carbon source and, as it has not been found to inhibit these bacteria, will

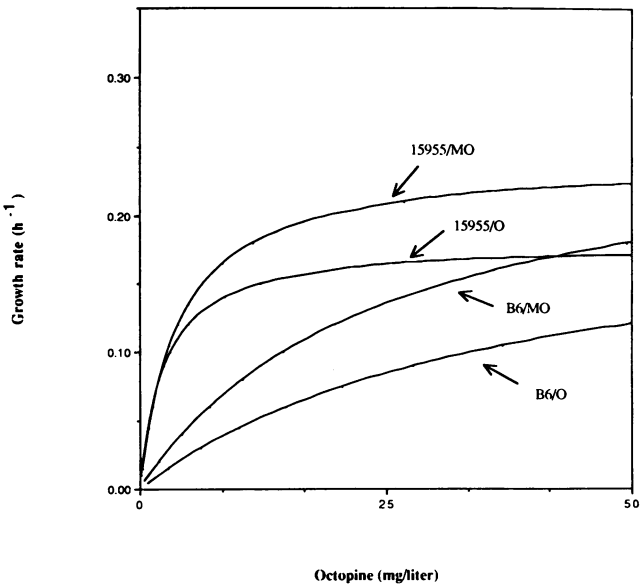

FIG. 2. Monod curves for B6 and ATCC 15955 with octopine as the limiting nitrogen source (MO) or limiting carbon source (O).

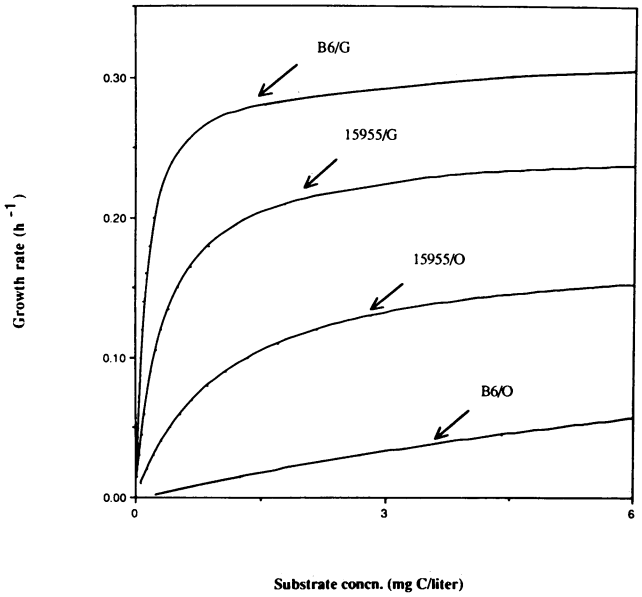

FIG. 3. Monod curves calculated on a carbon basis. G is glutamate medium; O is octopine medium.

not influence growth directly. Growth on glutamate-based media (Fig. 1) showed the difference between B6 and ATCC 15955; B6 used glutamate more efficiently than did ATCC 15955, both as a carbon source and as a nitrogen source. In both agrobacteria, glutamate was used more efficiently as the sole carbon source. Efficiency in this context means both the maximum specific growth rate under conditions of excess substrate and the growth possible under conditions of limiting substrate. Curves closer to the upper left corner of Fig. 1 to 4 are therefore deemed more efficient.

The situation was reversed in octopine-based media (Fig. 2). This time, ATCC 15955 was the better utilizer of substrate; in fact, B6 grew very poorly on octopine. In both strains, octopine was used more efficiently as the sole nitrogen source.

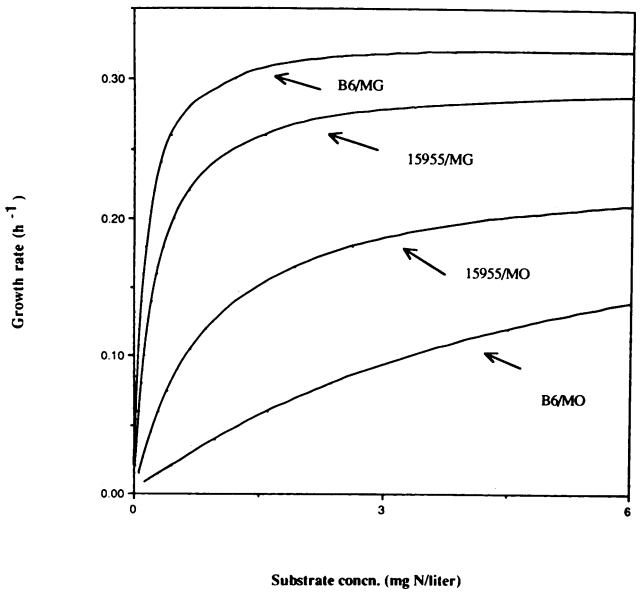

FIG. 4. Monod curves calculated on a nitrogen basis. MG is mannitol-glutamate medium; MO is mannitol-octopine medium.

The differences between agrobacteria and their growth on the amino acid substrates is most evident when K_S is expressed in milligrams of C or N per liter (Fig. 3 and 4). Both strains grew significantly faster on less substrate with glutamate than with octopine. B6 appeared to be the better grower on glutamate and the worst on octopine.

Yield coefficients in chemostats. The rate of growth and K_S do not always correlate with a microorganism's ability to synthesize biomass. Yield coefficients were determined to address this aspect (Table 2). Under conditions of carbon limitation by glutamate, yields were low for both strains (0.33 and 0.26). The yields under octopine limitation were reduced even further (0.12 and 0.16). A more valid comparison is achieved when both yields are converted to milligrams of C per liter. B6 growth on octopine showed a 2.5-fold reduction in yield compared with yields with growth on glutamate (0.81 to 0.34) and a fourfold reduction in ATCC 15955 yield (1.01 to 0.26). A significant decrease in yield did not occur between MG and MO. Yields per milligram of N on both nitrogen sources for both agrobacteria were comparable.

The deleterious effect of octopine as a carbon source was reflected in the low steady state biomass, as cell dry weight, and in the low cell productivity of the chemostat (Table 2). The total number of cells determined by epifluorescence microscopy in the octopine chemostats revealed another difference between B6 and ATCC 15955. ATCC 15955 produced significantly fewer cells when grown in O medium than in the other three media. Calculations of the average cell size (571 fg [dry weight] of cells) indicated that cells were much larger than cells grown in MG or G medium. Cell enlargement was also observed in MO medium. B6 did not produce larger cells in O medium; in fact, its value, 115 fg (dry weight) of cells, was the lowest encountered in all media.

Competition studies. A comparison of the Monod curves for these two agrobacteria in MG medium (Fig. 1 and 4) indicates that B6 should outcompete ATCC 15955 at all concentrations of glutamate. The simulation for the dual-culture chemostat at a dilution rate of 0.15 h^{-1} is shown in Fig. 5. The selection coefficient calculated from the slope of this line was 0.070. The slope for the observed competition was significantly higher at 0.117, demonstrating that B6 dominated the culture at a rate faster than that predicted by simple competition for limiting glutamate.

DISCUSSION

In assessing in vitro growth efficiency in these two agrobacteria, one needs to address both the μ_{max}/K_S interplay and the yield. Interesting differences between strains B6 and ATCC 15955 and between octopine and glutamate as substrates arise when such an analysis is performed. The observation that octopine, either as nitrogen or as the sole source of both carbon and nitrogen, produced lower μ_{max} values than glutamate with both agrobacteria is to be anticipated because of the extra catabolic steps required with octopine catabolism (50). In addition to the octopine permease, cleavage of octopine occurs by the action of an oxidase to form arginine and pyruvic acid. Dessaux et al. (9) have recently elaborated on the catabolism of arginine in *A. tumefaciens* and have shown that utilization of arginine as a carbon and nitrogen source proceeds through ornithine and proline to glutamate. If arginine is being assimilated solely

TABLE 2. Yield data for strains in continuous culture (dilution rate, 0.15 h^{-1})

Strain and growth medium	No. of cells/ml[a]	Cell dry wt (mg/liter)	Yield (mg [dry wt]/mg of glutamate)	Yield (mg [dry wt]/mg of octopine)	Yield (mg [dry wt]/mg of C)	Yield (mg [dry wt]/mg of N)	Cell productivity (mg [dry wt]/liter per h)	Avg cell size (fg [dry wt])
B6								
MG	1.5×10^9	269	0.82			10.90	26.9	183
G	7.5×10^8	163	0.26		0.81		22.8	217
MO	6.1×10^8	166		2.46		10.81	22.4	272
O	5.7×10^8	66		0.16	0.34		6.6	115
ATCC 15955								
MG	5.0×10^9	234	0.72			9.50	37.6	47
G	4.3×10^9	200	0.33		1.01		27.6	46
MO	2.0×10^8	151		1.44		6.34	21.2	757
O	9.0×10^7	51		0.12	0.26		6.7	571

[a] Acridine orange direct counts.

for nitrogen, two distinct pathways are operational. There is the formation of ammonia from an arginase/urease pathway and also the transamination of ornithine to Δ^1-pyrroline-5-carboxylate and then on to either proline or glutamate. This early distinction between pathways for carbon or nitrogen assimilation would also explain the observed differences in growth when octopine is used as a carbon or nitrogen source.

A most unexpected finding was the very high K_S of B6 for octopine as either the limiting carbon or nitrogen source. All other K_S values were of a magnitude common for bacteria (37). B6 has been domesticated and characterized for many years (18, 21); however, ATCC 15955 has been in culture collections even longer. It seems unlikely that this response of B6 is an accommodation to laboratory media; most workers do not routinely subculture on octopine! It would appear that although B6 is an extremely effective producer of crown galls on a wide range of plants (8), it is extremely inefficient at utilizing the octopine ultimately secreted from the gall.

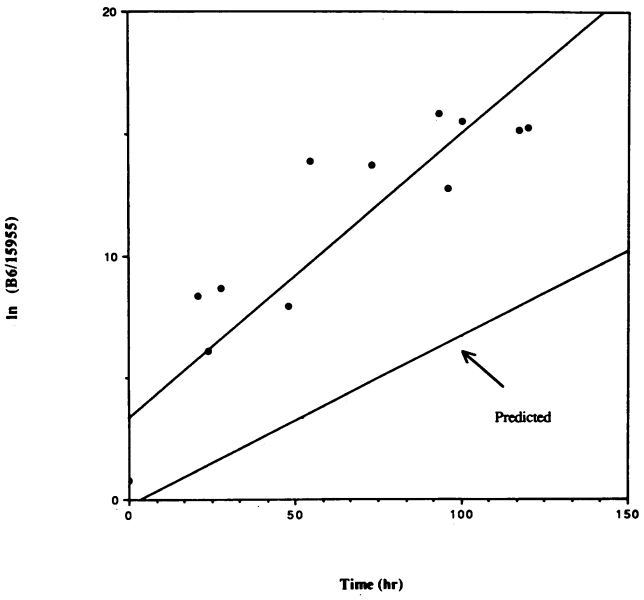

FIG. 5. Observed competition (●) of B6 and ATCC 15955 in MG medium under nitrogen limitation. The dilution rate was 0.15 h^{-1}. The regression line is $y = 3.31 + 0.117x$ ($r^2 = 0.813$). The regression line for the predicted competition is $y = -0.24 + 0.070x$ ($r^2 = 1.0$).

The data on yield coefficients suggest how octopine may be utilized in natural habitats. The carbon conversion efficiencies for the yield values per milligram of C in octopine media of 0.34 and 0.26 (Table 3), assuming an average percent carbon composition of a bacterial cell of 50% (1), translate to approximately 15%. The loss of 85% of a carbon substrate through respiration and excretion in a chemostat is significant. It is hard to envisage how an organism could tolerate this in the tumorosphere or rhizosphere. The opine niche would have to be a very exclusive one before an organism could prosper with these low yields and productivities. The corresponding analyses for nitrogen conversion ratios in MO medium (yield of 10.81 and 6.34, 10% nitrogen composition) produce figures of approximately 100% and 65%, respectively. The use of octopine as a nitrogen supplement by agrobacteria in natural habitats seems to be a more plausible event.

Extrapolations to in situ conditions must be made cautiously with chemostat data. Although it is an artificial growth vessel, it does provide sophisticated control for measuring growth parameters. Because it is an open system with input and output, it also approximates the exudation of opine from a gall and the leaching of that amino acid through the rhizosphere. The elevated numbers of microorganisms in the rhizosphere produce habitats in which crowding is intense and competition for resources exuded by the plant is fierce. The opine niche is no exception. A variety of agrobacterial biotypes, virulent and nonvirulent, are routinely isolated from plant galls and rhizospheres (5, 6, 22, 33, 42, 45, 47). Recent papers demonstrate that pseudomonads and coryneforms are also able to utilize opines (2, 39, 53). Even though such competitors for opines are present in the rhizosphere, the data for B6 and ATCC 15955 suggest that these agrobacteria are not able to respond to the appearance of octopine by a spurt of high growth rate. The fastest growth rate supported was 0.27 h^{-1} (mean generation time = 2.6 h). This is not sufficiently rapid to make a classic *r*-strategy response likely.

An appreciation of the fate of B6 and ATCC 15955 in direct competition with each other can be gleaned from an analysis of the growth parameters at hand. In an octopine-free environment, as represented by the Monod curves for MG and G medium, B6 will outcompete ATCC 15955 at all glutamate concentrations. The rate of growth produced by B6 on low concentrations of glutamate should allow it to compete favorably as a saprophyte.

A pertinent question to ask is this one: what advantage accrues to B6 if it is the transforming agent in a plant which

it has galled? Octopine is not the only opine excreted from galls; indeed, in some plants it appears to be a minor one in terms of quantity (43, 44). It could be that B6 will favor the catabolism of other opines, octopinic acid, lysopine, or agropine, and reserve octopine as an inducer of the *tra* region on the Ti plasmid (25).

If octopine is catabolized, because it will be utilized in addition to the other opines and numerous plant exudates, the dynamics will follow a multisubstrate pattern of use instead of the single-substrate one analyzed here. In multisubstrate regimens, yield is of significance (27, 48). The high yield of B6 on octopine would endow it with a competitive advantage in multisubstrate conditions over an octopine utilizer such as ATCC 15955. The rapidly changing conditions in the tumorosphere with respect to concentrations of opines and other exudates will favor the growth characteristics of one agrobacterium over another in time; a succession of types will result.

The parameters obtained for B6 and ATCC 15955 probably represent two sets of data from a continuum typical of virulent agrobacteria. The speculations on the ecological interpretation presented here suggest that there will be a dynamic community of opine utilizers, agrobacteria, and other genera which benefit from a crown-galled plant. The in vitro growth data presented here have provided some theoretical ideas to help us understand the ecological dynamics of microorganisms associated with galled tissue. This foundation can now be combined with in situ techniques to explore the ecology in growth chambers and greenhouse settings.

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