Single-Nutrient Microbial Competition: Qualitative Agreement Between Experimental and Theoretically Forecast Outcomes

Abstract. *When microbial strains compete for the same limiting nutrient in continuous culture, resource-based competition theory predicts that only one strain will survive and all others will die out. The surviving strain expected from theory will be the one with the smallest subsistence or "break-even" concentration of the limiting resource, a concentration defined by the* J *parameter. This prediction has been confirmed in the case of auxotrophic bacterial strains competing for limiting tryptophan. Because the value of* J *can be measured on the strains grown alone, the theory can predict the qualitative outcomes of mixed-growth competition in advance of actual competition.*

In the past 20 years a mechanistic theory of microbial competition has been under development (*1*), an extension of the theory of single-strain growth in continuous culture formulated independently by Monod (*2*) and Novick and Szilard (*3*). This theory generates a critical parameter J which, in principle, can be used to predict the surviving strain in mixed-strain culture on a single limiting nutrient. We now report specific experimental tests that support the J criterion as a means for successfully predicting the competitive outcome when the limiting resources are known.

For two competing strains grown in mixed continuous culture, a laboratory idealization of an environment with a constant carrying capacity, the equations of growth are

$$\frac{dS}{dt} = (S_0 - S)D - \frac{\mu_1}{y_1}\frac{S \cdot N_1}{K_{s_1} + S} - \frac{\mu_2}{y_2}\frac{S \cdot N_2}{K_{s_2} + S}$$

$$\frac{dN_1}{dt} = \frac{\mu_1 S \cdot N_1}{K_{s_1} + S} - D \cdot N_1$$

$$\frac{dN_2}{dt} = \frac{\mu_2 S \cdot N_2}{K_{s_2} + S} - D \cdot N_2 \qquad (1)$$

where S is the concentration of the one limiting nutrient in the culture (all other nutrients supplied in excess of demand), S_0 is the input concentration of the limiting nutrient, and D represents the influent and effluent rates of medium. For the ith organism, N_i is the concentration of cells in the culture, D is the death rate due to cell outflow, μ_i is the maximum per cell division (birth) rate, y_i is the yield (cells per unit of nutrient), and K_{s_i} is the half-saturation constant for the limiting resource (*4*).

Hsu *et al.* (*5*) have mathematically analyzed the global asymptotic behavior of Eq. 1 and its extension to an arbitrary n competing species or strains. They have proved that any system governed by the n-species generalization of Eq. 1 will approach a globally stable equilibrium, in which either (i) all competitors die out ("washout"), or else (ii) one species survives (*6*). Which species survives, or whether total washout occurs, depends on S_0 and on the J parameters for each species or strain. For the ith species, the J parameter is

$$J_i = K_{s_i}\left(\frac{D}{r_i}\right) \qquad (2)$$

where $r_i = (\mu_i - D) > 0$, the intrinsic rate of increase of the ith species. With no loss of generality, number the species such that their J's are ordered, $J_1 < J_2 < \ldots < J_n$. Total washout occurs if $J_1 > S_0$, such that lim $N_i = 0$, $i = 1, \ldots n$, and lim $S = S_0$. However, if $J_1 < S_0$, then species 1 survives and outcompetes all rival species, such that lim $N_1 = y_1(S_0 - J_1)$, lim $N_i = 0$, $i = 2, \ldots n$, and lim $S = J_1$. These results have been extended to cases of unequal death rates, in which case the D's are subscripted for each species in Eqs. 1 and 2 (*7*). The parameter J_i defines the subsistence concentration of the limiting resource for the ith species, and the steady-state concentration of the resource when ith species is grown alone.

The J criterion for competitive ability is nonobvious and requires experimental verification. It could not have been predicted from classical theories of competition (*8*). A priori it might have been expected that the winner would always be the species with the highest affinity (lowest K_s) for the nutrient, or perhaps the organism with the highest intrinsic rate of increase; in fact there are conflicting opinions on this question (*9*). However, the extended theory of Monod and of Novick and Szilard asserts that it is actually a weighted K_s value which is critical to competitive success—weighted by the ratio of the death rate to intrinsic rate of increase. Thus, a species with a higher affinity for the resource may nevertheless lose if it also has a lower intrinsic rate or higher death rate. The theory also asserts that winning will be independent of the growth efficiency (yield) of the species grown on the limiting resource.

To make a rigorous test of the J criterion in continuous culture requires proof that (i) if two strains have equal r's and D's, the strain with the lower K_s wins; (ii) if two strains have identical K_s's and D's, the strain with the higher r wins; and (iii) if two strains have different K_s's and r's, but in spite of this still have identical J's, then the species or strains will coexist indefinitely. We have conducted all three of these tests with auxotrophic bacterial strains that require an exogenous source of tryptophan for growth. The competition experiments were conducted in two parts. First, the K_s and μ parameters were measured for each bacterial strain grown alone in batch culture

Table 1. Uptake and growth parameters for competing bacterial strains.

Experiment No.	Bacterial strain	Auxotrophic for tryptophan					Other run parameters		
		Yield (cell/g)	K_s (g/liter)	μ (per hour)	r (per hour)	J (g/liter)	S_0 (g/liter)	D (per hour)	Volume (ml)
1	C-8*	2.5×10^{10}	3.0×10^{-6}	0.81	0.75	2.40×10^{-7}	1×10^{-4}	6.0×10^{-2}	200
	PAO283†	3.8×10^{10}	3.1×10^{-4}	0.91	0.85	2.19×10^{-5}			
2	C-8 nal^{r}spec^s	6.3×10^{10}	1.6×10^{-6}	0.68	0.61	1.98×10^{-7}	5×10^{-6}	7.5×10^{-2}	200
	C-8 nal^{s}spec^r	6.2×10^{10}	1.6×10^{-6}	0.96	0.89	1.35×10^{-7}			
3‡	C-8 nal^{r}spec^s	6.3×10^{10}	1.6×10^{-6}	0.68	0.61	1.98×10^{-7}	5×10^{-6}	7.5×10^{-2}	200
	C-8 nal^{s}spec^r	6.2×10^{10}	0.9×10^{-6}	0.41	0.34	1.99×10^{-7}			

**Escherichia coli.* †*Pseudomonas aeruginosa.* ‡Nalidixic acid added (0.5 μg/ml).

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on limiting tryptophan (*10*). From these measurements, the values of J were calculated to predict the outcomes of subsequent competition experiments, the second part of each test (*11*).

Growth parameters for the strains grown alone are presented in Table 1. In the first experiment, *Escherichia coli* strain C-8 was opposed by *Pseudomonas aeruginosa* strain PAO283. The two strains differ in their tryptophan K_s's by approximately two orders of magnitude. As a result, the J value of C-8 is much smaller than the J value for PAO283. Theory predicts that strain C-8 should be the winner when the two strains are grown together on limiting tryptophan. This prediction was qualitatively correct (Fig. 1a), although the actual time course of competitive exclusion deviated quantitatively to some extent from that predicted by Eq. 1 (*12*). Strain C-8 effectively eliminated PAO283 in about 60 hours, in spite of the fact that PAO283 had a higher r and a starting numerical advantage of 200 : 1.

The first experiment affirms the importance of having high affinity (low K_s) for the limiting nutrient; the second experiment demonstrates that competitive outcomes are not solely determined by K_s, but also depend on the intrinsic rates of increase. Competition occurred between two F^- clones of *E. coli* strain C-8. One line was selected to be resistant to the metabolic inhibitor nalidixic acid, but sensitive to spectinomycin ($nal^r spec^s$); and the other strain was selected to have the reverse pattern of resistance. After selection, both strains had the same K_s values, but different μ's and J's (Table 1), from which C-8 $nal^s spec^r$ is the predicted winner. This qualitative prediction was again correct (Fig. 1b), although the losing strain had a faster death rate than expected from Eq. 1 (*12*). Again the strain expected to lose was given a starting numerical advantage, but the outcome was unaffected.

The third experiment demonstrates that it is critically the weighted value of K_s, given by J, which is the determining factor. It proves that coexistence occurs when the J's are equal despite differences in the half-saturation constants and intrinsic rates of increase. We determined that in the presence of small amounts of nalidixic acid in the culture medium, both the K_s and μ parameters of C-8 $nal^s spec^r$ were altered. Within the range of 0.2 to 0.8 μg/ml, the growth rate of C-8 $nal^s spec^r$ was linearly depressed by increasing nalidixic acid concentrations, whereas the growth rate of C-8 $nal^r spec^s$ was hardly affected (Fig. 1c). With the addition of nalidixic acid (0.5 μg/ml), K_s and r were such as to make the J's of the two strains identical (Table 1). Theory in this case predicts coexistence; our experimental results are consistent with this prediction (Fig. 1d) (*13*).

These tests of the extended theory of Monod and of Novick and Szilard are instructive in interpreting the findings of other studies of mixed-strain microbial growth. A number of workers studying single-nutrient competition in continuous culture have reported competitive reversals depending on the flow rate, D, and the input concentration, S_0 (*14*). Reversals are predicted by the theory to occur only if the strain with the smaller K_s value also has the smaller maximum specific growth rate, μ. However, the "re-

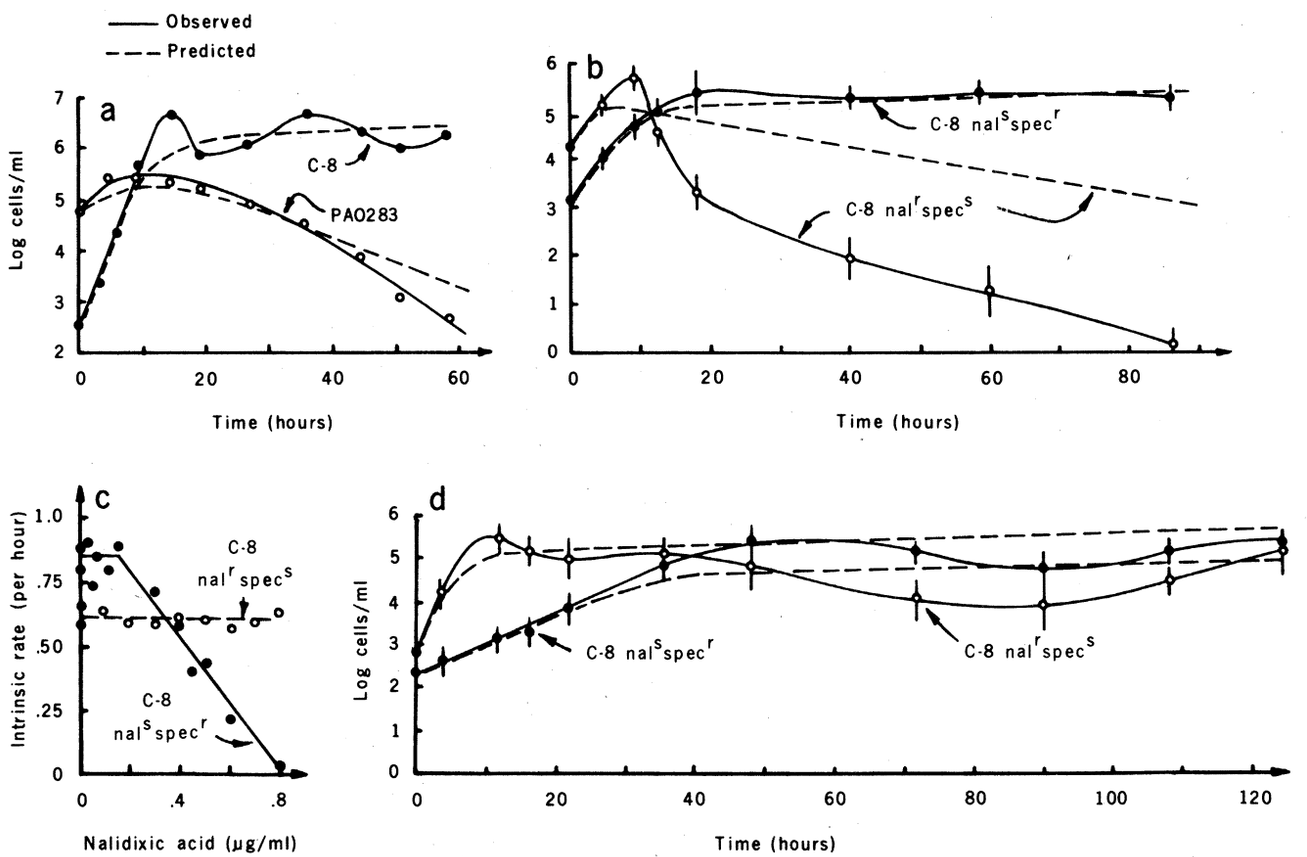

Fig. 1. (a) Experiment 1: Strains differ principally in their half-saturation constants for tryptophan, and PAO283 loses to C-8 as predicted. (b) Experiment 2: Strains differ in their intrinsic rates of increase, but not in their half-saturation constants, and C-8 $nal^r spec^s$ loses to C-8 $nal^s spec^r$ as predicted. (c) Effect of nalidixic acid on intrinsic rate of increase of strains C-8 $nal^s spec^r$ and C-8 $nal^r spec^s$. (d) Experiment 3: Strains differ in the half-saturation constants and in their intrinsic rates of increase, but nevertheless have identical J parameters, and the strains coexisted for the duration of the experiment, as predicted. In each experiment, the predicted curves were obtained by numerical integration of Eq. 1. Bars around points in experiments 2 and 3 (b and c) indicate ranges of three replicate values.

versal" of the surviving strain is predicted, not because of competition, but because the strain with the lower K_s and μ is washed out at the higher flow rate, whereas the strain with the higher μ is not. Consequently, it must be shown that both species grown alone can persist at the given flow rate to establish that competition was the agent of elimination. For a given D, this is equivalent to showing that $r > 0$ for both strains.

Reversals are not predicted by the theory for variation in the influent concentration of the limiting resource, S_0 (*5, 7*). If such reversals occur, it is probable that at higher levels of S_0 the culture has become limited by some other nutrient for which a strain different from the previous winner now has the lower J. The equations of mixed growth (Eq. 1) are then altered to describe the consumption of the new limiting resource, R, with concomitant changes in the growth parameters of each strain (K_s, μ, y) that are appropriate for the new resource. Accordingly, the number of strains potentially capable of winning in mixed-growth culture depends (i) on the number of potentially limiting resources in the culture medium and (ii) on the distribution of minimal J's for these resources among the competing strains. Thus, where there are more potentially limiting resources than strains, and all strains have at least one minimal J for some resource, then all strains are potential winners with appropriate choices of resources.

When limiting resources are known, resource-based competition theory represents a conceptual advance over classical theory because the outcomes of exploitative competition can be forecast from data taken on the rival species grown alone. Thus, for two competing species with similar death rates, the winner is expected to be the species whose half-saturation constant for the limiting resource is smaller in comparison to its intrinsic rate of increase. A good competitor is a species able to grow at low resource concentrations at a higher intrinsic rate than its rivals, an ability summarized in the parameter J. Classical competition theory asserts that two-species outcomes are independent of the intrinsic rates of increase of the two species (*15*), a claim that the more mechanistic resource-based approach shows to be incorrect.

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References and Notes

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2. J. Monod, *Ann. Inst. Pasteur (Paris)* **79**, 390 (1950).
3. A. Novick and L. Szilard, *Science* **112**, 715 (1950).
4. The elementary theory of pure-strain growth in continuous culture has been the subject of many experimental and theoretical investigations, as reviewed by S. J. Pirt, *J. Appl. Chem. Biotechnol.* **22**, 55 (1972).
5. S. B. Hsu, S. Hubbell, P. Waltman, *SIAM (Soc. Ind. Appl. Math.) J. Appl. Math.* **32**, 366 (1977).
6. F. M. Stewart and B. R. Levin (*1*) have shown that the conclusions hold for a more general set of assumptions than those in Eq. 1, via linearized analysis near equilibrium points.
7. S. B. Hsu, *SIAM (Soc. Ind. Appl. Math.) J. Appl. Math.*, in press.
8. In classical competition theory, attributed to A. J. Lotka and V. Volterra, competitive outcomes depend on relationships between "carrying capacities" (equilibrium population sizes when each species is grown alone) and "competition coefficients" (per capita effects by each species on the growth rate and equilibrium population of rival species). This is a phenomenological theory in the sense that it seeks to describe how populations of competing species change without being specific about which resources are limiting and the focus of competition, nor about the relative abilities of the species to exploit these resources. Parameters such as S_0, K_s, μ, and y do not appear. Application of classical theory for prediction has been hampered by the difficulty of measuring the competition coefficients independently of actually growing the competitors together.
9. The most frequently expressed opinion is that the K_s values should be sufficient to explain the competitive outcomes between microorganisms [for example, R. W. Eppley and J. L. Coatsworth, *J. Phycol.* **4**, 151 (1968); P. Kilham, *Limnol. Oceanogr.* **16**, 10 (1971)]. D. Titman [*Science* **192**, 463 (1976); *Ecology* **58**, 338 (1977)] performed a direct test of resource-based competition theory between two diatom species, *Cyclotella* and *Asterionella*. In competition, *Cyclotella* wins under silicate limitation, and *Asterionella* wins under phosphate limitation. Titman's results are consistent with the J criterion.
10. A series of flasks containing different amounts of tryptophan in 100 ml of minimal medium was placed in a constant temperature shaker bath at 34°C. Initial cell density was kept very low (10^3 cell/ml); tests showed that no detectable alteration in batch tryptophan concentration occurred at these cell densities during the 8-hour determinations. Each hour for 8 hours, samples were removed, plated, and counted after 24 hours of incubation. Microscopic cell counts confirmed plate counts and a lack of cell clumping. Regression of ln (cells per milliliter) against time gave the log-phase growth rate at each concentration of tryptophan.
11. Competition experiments were carried out in autoclavable, continuous flow chemostats (VirTis) at a culture volume of 200 ml, in a constant temperature shaker bath at 34°C. To monitor growth of each strain, samples were withdrawn under conditions of sterility, diluted to a range of known concentrations of the original sample, and plated. Sequential serial dilution was necessary to ensure that accurate plate counts could be made, regardless of the cell density of the strain at the time of sampling (densities ranged over four to six orders of magnitude in the culture). Each strain was counted on two separate series of plates treated with an inhibitor to which one strain was resistant and the other sensitive. Microscopic cell counts confirmed that plate counts were accurate. No measurable wall growth or cell clumping occurred at the low cell densities reached in culture.
12. In both experiments 1 and 2, death rates of the losing strain were faster than predicted by Eq. 1. This was most likely due to the existence of a minimum threshold tryptophan concentration below which the losing strain experiences an increased death rate. Concentrations of tryptophan are driven below this threshold by the winning strain.
13. The cause of the fluctuations in the two strains at approximate steady state is not known, but occurred in all three replicate cultures at about the same time. The pumping rate may have been affected by fluctuations in line voltage.
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15. C. Strobeck, *Ecology* **54**, 650 (1973).
16. We thank N. Miles and L. Diament for help in the experiments, and Drs. Erich Six and Allen Markovitz for supplying the original bacterial stocks. This research was supported by the National Science Foundation.

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Motile Flagellum with a "3 + 0" Ultrastructure

Abstract. *The male gamete of the parasitic protozoan* Diplauxis hatti *has a flagellum consisting of three doublet microtubules. This flagellum exhibits a helicoidal waveform in which bends propagate toward the tip with a frequency of about 1.5 hertz. It is the simplest motile eukaryotic flagellum yet described.*

Most flagella and cilia of eukaryotic cells have a classical "9 + 2" axoneme in which a pair of single microtubules is surrounded by a ring of nine doublet microtubules (*1*). A number of exceptions to this general scheme have been found, but generally the motility of the cells has not been described (*2*). The simplest motile axoneme reported so far is the 6 + 0 flagellum of the male gamete of a parasitic protozoan, the gregarine *Lecudina tuzetae* (*3*). The existence of an axoneme consisting of only three doublets has been noted in the male gamete of another gregarine, *Diplauxis hatti* (*4*); we now report that this axoneme is motile, and describe its structure and motility.

The male gamete of *Diplauxis hatti* is a spherical cell about 4 μm in diameter (Fig. 1a), with a flagellum about 20 μm long. The diameter of the flagellum is only 0.11 to 0.13 μm, compared to the typical diameter of 0.2 μm for classical cilia and flagella (*1*). The flagellum runs along the nucleus for several micrometers before emerging from the cell body. The axoneme has a 3 + 0 microtubular structure both in the flagellum (Fig. 1b) and within the cell body (Fig. 1c).

The ultrastructure of the doublets is similar to that of the doublets from a 9 + 2 flagellum; they measure about 34 by 26 nm. If the doublets are oriented with the A-subfibers facing clockwise

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