The Influence of the Growth Environment on the Stability of a Drug Resistance Plasmid in *Escherichia coli* K12

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Populations of *Escherichia coli* K12 containing the plasmid TP120 which coded for resistance to ampicillin, streptomycin, sulphonamide and tetracycline were grown in a chemostat under carbon-limited and phosphorus-limited conditions. With time, resistance to one or more of the drugs was lost, resulting in the production of mutant populations which were more competitive than the parent population. The resistance to tetracycline was always lost under both carbon and phosphorus limitations, but resistance to the other three drugs was lost only during phosphate-limited growth. Strains of *E. coli* which had lost resistance to one or more of the drugs were capable of higher maximum specific growth rates than the parent strain.

INTRODUCTION

Plasmids coding for drug resistances have been extensively investigated in terms of their biochemical, genetic and epidemiological properties but relatively little is known about the behaviour of plasmids and plasmid-carrying organisms under different environmental and physiological conditions. In particular, the effect of different growth environments and host growth rates on the survival and stability of extrachromosomal elements within a population has only recently been considered (Melling *et al.*, 1977). Experiments with R factor-carrying *Escherichia coli* in the human gut have shown that the antibiotic-resistant population disappeared rapidly which suggested that the plasmid-containing population was less competitive than the resident populations (Anderson, 1974; Anderson, E. S., 1975). Fresh challenges with antibiotics resulted in the re-establishment of the resistant population (Anderson *et al.*, 1973). Under non-challenge conditions it was suggested that the loss of the resistant population might be due to impaired growth rates of the plasmid-containing population compared with the plasmid-minus population (Anderson, 1974) and/or inhibition of plasmid transfer by bile salts, inert bacterial material and the density of the competitive organisms (Anderson, J. D., 1975). Conversely, in water supplies and raw sewage, drug-resistant coliforms showed a long-term survival and capacity to transfer the resistance factors in mixed populations (Grabow *et al.*, 1974). Antibiotic-resistant Enterobacteriaceae occur at high frequencies in aquatic environments and it has been suggested that they survive well under these conditions (Smith, 1970; Freary *et al.*, 1972; Hughes & Meynell, 1975).

A number of closed culture studies have all shown that plasmid-containing microbial populations had lower maximum specific growth rates than plasmid-minus populations (Terawaki *et al.*, 1968; Yokota *et al.*, 1972; Grinsted & Lacey, 1973; Lacey, 1975; Kayama & Yara, 1975; Inselburg, 1978; Nakazawa, 1978). This strongly suggests that plasmid-containing populations are at a competitive disadvantage under growth conditions where the characteristics coded for by the plasmid are not or do not need to be expressed. Melling *et al.* (1977), using chemostat continuous-flow culture techniques, showed that *E. coli*

containing plasmid RP1 competed unsuccessfully against a plasmid-minus isogenic strain at submaximal growth rates. These results indicated that under appropriate growth conditions a competitive plasmid-minus population ought to be selected for from the original plasmid-containing parent population and in an open growth system replace the parent population. However, Melling *et al.* (1977) failed to select for a plasmid-minus population from a parent RP1-containing strain of *E. coli* under a number of different growth limitations, including carbon, magnesium and phosphate limitations. Conversely, an F' *lac*-carrying *E. coli* population growing under lactose-limited conditions in a chemostat was replaced after a short period by a more competitive F⁻ strain derived from the parent strain (Slater & Bull, 1978).

This study was undertaken to investigate the effects of various growth conditions (carbon and phosphorus limitation) and growth rates, using continuous-flow culture techniques, on the stability of a plasmid coding for resistance to ampicillin, streptomycin, sulphonamide and tetracycline in *E. coli* K12 in the absence of any of the drugs.

METHODS

Bacterial strains and plasmids. *Escherichia coli* K12 F⁻ strain 1R713 was kindly supplied by G. Willshaw (Central Public Health Laboratory, Colindale, London). This strain, designated K12(TP120), contained the plasmid TP120 (R46 or R Brighton) (Grindley *et al.*, 1973) of compatibility group N which carried resistance to ampicillin (Ap), streptomycin (Sm), sulphonamide (Su) and tetracycline (Tc) and was auto-transferable. During the continuous-flow culture selection experiments, the following resistant strains were isolated: from a carbon-limited chemostat, K12(TP120A) (Ap, Sm, Su); and from phosphate-limited chemostats, K12(TP120B) (Sm, Su, Tc), K12(TP120C) (Ap, Sm, Su) and K12(TP120D) (Sm, Su). The nalidixic acid-resistant strain of *E. coli* K12 711 NaI^R [J62 nal-r, Harden & Meynell (1972)] used as a recipient in transfer experiments was provided by K. P. Flint (University of Warwick).

Culture maintenance and growth. The strains of *E. coli* were maintained on nutrient agar slopes containing appropriate drugs at the following concentrations ($\mu\text{g ml}^{-1}$): ampicillin, 10; streptomycin, 10; sulphonamide, 50; tetracycline, 10. For closed culture growth, the organisms were grown in nutrient broth or the minimal medium described by Davis & Mingioli (1950) containing 10% (w/v) glucose or Hershey's glucose mineral salts medium (Hershey, 1955). The cultures were incubated at 37 °C with vigorous aeration and growth was measured by determining the absorbance (A_{520}) in a Corning model 252 colorimeter. The Davis & Mingioli minimal medium pH 7.0, supplemented with glucose to a growth-limiting concentration of 0.2 g l⁻¹, was used for glucose-limited continuous-flow culture. For phosphate-limited chemostat growth, the organisms were grown in Hershey's glucose mineral salts medium (Hershey, 1955) at pH 7.4 with phosphate at 0.005 g l⁻¹. The continuous-flow culture vessels had a working volume of 1 l and were agitated at 1000 rev. min⁻¹ with air supplied at 1 l min⁻¹; the growth temperature was 37 °C.

Procedure for selection and competition experiments in continuous-flow culture. For selection experiments, chemostats were inoculated with 10 ml of an overnight culture of *E. coli* K12(TP120) and grown in batch culture for several hours in medium containing the drugs at the same concentration as the stock slopes. The fresh medium flow was initiated to give the required dilution rate and initially a steady state culture was established in the presence of the drugs. The medium was then changed to one lacking the drugs and samples were taken at 24 h intervals for viable organism determination. After appropriate dilution in 0.1 M-KH₂PO₄/KOH buffer pH 7.0 at 37 °C, 0.1 ml samples were plated on nutrient agar plates, glucose minimal medium agar plates and glucose minimal medium agar plates containing, separately, at 60% minimal inhibitory concentration ($\mu\text{g ml}^{-1}$): ampicillin, 50; streptomycin, 10; sulphonamide, 10; or tetracycline, 25. Viable counts were determined after 2 d at 37 °C.

Two-membered mixed culture competition experiments were performed with *E. coli* K12(TP120) and *E. coli* K12(TP120A). Separate overnight cultures grown in glucose mineral salts medium were simultaneously inoculated into a glucose-limited chemostat to give approximately equal population densities. After a period of closed culture growth, to overcome problems associated with variable lag phases and to ensure that both populations were growing exponentially, the fresh medium flow was initiated to give the selected dilution rate, ranging from 0.10 to 0.35 h⁻¹. Samples were taken at 3 to 4 h intervals for viable organism determination by plating on nutrient agar and nutrient agar supplemented with tetracycline at 25 $\mu\text{g ml}^{-1}$. Viable counts were determined after 2 d at 37 °C. The specific growth rate of the uncompetitive population, μ_1 , was calculated in two ways. Firstly, using a modified washout growth rate equation due to Jannasch (1969):

$$\mu_1 = \frac{\ln x_t - \ln x_0}{(t_2 - t_1)} + D \quad (1)$$

where x_0 is the initial uncompetitive population size at time t_1 , x_t is the final uncompetitive population size at time t_2 and D is the dilution rate maintained for the duration of the experiment. Secondly, the difference in the specific growth rates of the two competing populations was calculated from:

$$(\mu_1 - \mu_2) = \frac{\ln R_t - \ln R_0}{(t_2 - t_1)} \quad (2)$$

where R_0 is the initial ratio of the competitive population to the uncompetitive population at time t_1 , R_t is the final ratio of the two populations at time t_2 , μ_1 is the specific growth rate of the uncompetitive population x_1 and μ_2 is the specific growth rate of the competitive population x_2 . When $x_2 \gg x_1$, then in chemostat culture $\mu_2 = D$ (Slater & Bull, 1978) and so:

$$\mu_1 = \frac{\ln R_t - \ln R_0}{(t_2 - t_1)} + D \quad (3)$$

Isolation of plasmid DNA. Plasmid DNA was isolated by a dye buoyant caesium chloride density gradient centrifugation method (Palchaudhuri & Chakrabarty, 1976) as modified by K. G. Hardy (University of Kent, personal communication).

Conjugation. The maintenance, or otherwise, of an autotransferable plasmid in the various isolates obtained by continuous-flow culture selection was demonstrated by showing its potential to transfer to a plasmid-minus recipient (Dennison & Baumberg, 1975). Cultures of the recipient were grown in nutrient broth to the end of the exponential phase giving approximately 5×10^9 organisms ml^{-1} ; the donors were grown to mid-exponential phase in either nutrient broth or the minimal media, giving approximately 2×10^8 organisms ml^{-1} . Recipient *E. coli* Nal^R (0.1 ml) was spread on nutrient agar plates supplemented with 10 μg ampicillin ml^{-1} or 10 μg sulphonamide ml^{-1} . The donor strains were serially diluted separately to 10^{-6} dilution and 0.02 ml of each dilution was inoculated dropwise on to the surface of the plates spread with *E. coli* Nal^R. The plates were incubated for 18 h at 37 °C and the number of colonies growing at a suitable dilution were counted. The colonies represented nalidixic acid-resistant *E. coli* organisms which had received by conjugation a plasmid carrying ampicillin or sulphonamide resistance (transconjugants), and the frequency of transfer was expressed as the percentage of transconjugants to total donor organisms. For each conjugation experiment, a number of transconjugant colonies were transferred to a range of media supplemented separately with the four drugs to check for the concomitant transfer of all the expected resistance markers.

RESULTS

Plasmid stability and strain selection

The stability of populations of *E. coli* containing plasmid TP120 was examined under glucose-limited and phosphate-limited conditions at dilution rates between 0.10 and 0.35 h^{-1} . In all experiments, at least one of the drug resistance markers was eventually eliminated from the population. However, the pattern of loss of markers from glucose-limited cultures was different from that from phosphorus-limited cultures. In all the glucose-limited cultures only the resistance to tetracycline was lost. In the experiment shown in Fig. 1, the parent population, as measured by the loss of tetracycline resistance, declined by a factor of more than 10^6 between 500 and 800 h after changing to drug-free medium; it was replaced by a second population, *E. coli* K12(TP120A), lacking resistance to tetracycline but retaining resistance to ampicillin, streptomycin and sulphonamide. For a further 1000 h of growth there was continuous presence of the parent population at concentrations varying between 10 and 10^3 organisms ml^{-1} (up to 0.0001 % of the total population). During this time no additional changes in the dominant population were apparent and there was no further loss of drug resistance markers. The dominant population from the selection experiment at $D = 0.10 \text{ h}^{-1}$, *E. coli* K12(TP120A), contained a plasmid, as shown by density gradient centrifugation following cell lysis, and retained the capacity to transfer a plasmid carrying resistance to ampicillin, streptomycin and sulphonamide (Table 1). The other strains showing similar phenotypes to *E. coli* K12(TP120A) that were isolated from selection experiments at higher dilution rates were not analysed further.

Fig. 1. The growth of *E. coli* K12(TP120) in a glucose-limited chemostat at a dilution rate of 0.10 h^{-1} illustrating the displacement of the tetracycline resistance marker (and hence the parent population) and the domination of a derivative strain [*E. coli* K12(TP120A)] resistant to ampicillin, streptomycin and sulphonamide but sensitive to tetracycline. Time 0 is the time at which drug-free medium flow was started. Total number of viable organisms (○), and number of viable organisms resistant to tetracycline (●), ampicillin (□), streptomycin (■) and sulphonamide (△).

Table 1. *Presence of plasmids in selected E. coli* K12 strains and frequencies of plasmid transfer

The presence of plasmids was examined by density gradient centrifugation. Plasmid transfer ability was tested with the plasmid-minus recipient *E. coli* K12 Nal^R. Strains were derived from continuous-flow selection experiments at a dilution rate of 0.10 h^{-1} under carbon-limited conditions (a) or phosphorus-limited conditions (b).

<i>E. coli</i> K12 with plasmid (resistance markers)	Presence of plasmid	10 ³ × Percentage frequency of transfer of markers		
		Nutrient broth	Davis & Mingioli defined medium	Hershey defined medium
TP120 (Ap, Sm, Su, Tc)	+	5.5	5.3	1.8
TP120A ^a (Ap, Sm, Su)	+	5.0	0.1	0.3
TP120B ^b (Sm, Su, Tc)	+	1.4	0.1	0.6
TP120C ^b (Ap, Sm, Su)	+	0	0	0
TP120D ^b (Sm, Su)	+	0	0	0

A more complex pattern of loss of drug resistance markers associated with an increase in parent strain instability was observed in the phosphate-limited selection experiments. In common with selection under carbon-limited conditions, the tetracycline marker was routinely lost from steady state cultures at similar washout rates, but it was never completely eliminated and replaced by a more competitive tetracycline-minus mutant as might be expected theoretically. In only one instance under phosphate-limited conditions at

Fig. 2

Fig. 3

Fig. 2. The growth of *E. coli* K12(TP120) in a phosphate-limited chemostat at a dilution rate of 0.10 h^{-1} showing the loss of the tetracycline resistance marker and hence the parent population. The replacement population [*E. coli* K12(TP120C)] was resistant to ampicillin, streptomycin and sulphonamide, and all three resistances were individually present at above 10^8 resistant organisms ml^{-1} for the duration of the experiment. Time 0 is the time at which drug-free medium flow was started. Total number of viable organisms ($\circ$), and number of viable organisms resistant to tetracycline ($\bullet$).

Fig. 3. The growth of *E. coli* K12(TP120) in a phosphate-limited chemostat at a dilution rate of 0.35 h^{-1} showing the loss of ampicillin and tetracycline resistance markers. Time 0 is the time at which drug-free medium flow was started. Total number of viable organisms ($\circ$), and number of viable organisms resistant to tetracycline ($\bullet$) and ampicillin ($\square$).

$D = 0.10 \text{ h}^{-1}$ was tetracycline resistance the sole marker lost (Fig. 2). In this experiment the original population resistant to the four drugs persisted at a level of approximately 10 organisms ml^{-1} in a total population of 4×10^8 *E. coli* K12(TP120C) organisms ml^{-1} . Throughout the experiment all the other three drug resistances were individually shown to be present at concentrations above 10^8 resistant organisms ml^{-1} demonstrating that the disappearance of tetracycline resistance reflected only the washout of the parent population. Unlike the loss of tetracycline-resistant organisms under glucose-limited conditions, however, with phosphate as the growth-limiting nutrient the disappearance of the tetracycline resistance marker usually began soon after the removal of the drugs from the growth environment (Fig. 2), although this was not always the case (Fig. 3). A number of different selection experiments yielded dominant derivative strains with the same phenotype as *E. coli* K12(TP120C), but only the strain isolated at $D = 0.10 \text{ h}^{-1}$ was analysed and shown to contain a plasmid (Table 1). Unlike the comparable strain isolated from carbon-induced selection [*E. coli* K12(TP120A)], numerous attempts failed to demonstrate any retention of the capacity to transfer the derived plasmid into the recipient strain (Table 1).

An important difference between carbon-limited and phosphorus-limited stability and selection experiments (with the single exception of the phosphate-limited selection experiment just mentioned) was that under phosphate-limited conditions drug resistance markers other than tetracycline were also lost which led to the selection of different dominant populations with various plasmid genotypes in different experiments. Furthermore, these experiments produced mixed cultures with two or more different strains containing various

Table 2. *Maximum specific growth rates for selected E. coli K12 strains carrying various drug resistance markers (or none) in closed culture on defined media*

Strains were derived from continuous-flow selection experiments at a dilution rate of 0.10 h^{-1} under carbon-limited conditions (a) or phosphorus-limited conditions (b).

<i>E. coli</i> K12 with plasmid (resistance markers)	Maximum specific growth rate, $\mu_{\max} (\text{h}^{-1})$		Average percentage decrease of parent $\mu_{\max}$ compared with mutant strains
	Davis & Mingioli defined medium	Hershey defined medium	
TP120 (Ap, Sm, Su, Tc)	0.40	0.39	—
TP120A ^a (Ap, Sm, Su)	0.56	0.50	25
TP120B ^b (Sm, Su, Tc)	0.46	0.60	25
TP120C ^b (Ap, Sm, Su)	0.57	0.48	24
TP120D ^b (Sm, Su)	0.47	0.56	23
No plasmid ^b	0.69	0.90	50

combinations of drug resistance markers. For example, in one selection at $D = 0.10 \text{ h}^{-1}$, after 300 h growth in drug-free medium the culture was dominated by *E. coli* K12(TP120D) which carried resistance only to sulphonamide and streptomycin and contained a non-transferable plasmid (Table 1). After this period of growth the culture also contained a population only lacking resistance to tetracycline [that is, showing the same phenotype as *E. coli* K12(TP120A) and K12(TP120C)] at 0.001 % of the total population and a second population lacking resistance to ampicillin at 1 % of the total population. The latter strain was isolated, designated *E. coli* K12(TP120B) and shown to carry resistance to streptomycin, sulphonamide and tetracycline and to contain a transferable plasmid (Table 1).

Another feature of populations growing with phosphate as the limiting nutrient was that the washout curves for the loss of individual resistance markers rarely followed the anticipated washout kinetics (Fig. 3). The change in the logarithm of the population(s) with resistance to a specific drug was usually non-linear, exhibited plateau periods and occasionally short phases of transient increase in drug resistance before washout continued (Fig. 3).

Competition between the parent organism, E. coli K12(TP120) (Ap, Sm, Su, Tc), and a tetracycline-sensitive organism, E. coli K12(TP120A) (Ap, Sm, Su), in open culture

The continuous-flow culture stability experiments showed that, particularly under phosphate-limited growth conditions, it was possible to select for mutant strains carrying decreased numbers of drug resistance markers. Indeed, the final stage of one phosphate-limited stability experiment yielded an organism lacking resistance to all four of the drugs and which could not be shown to contain a plasmid. Furthermore, in prolonged selection experiments, the derivative strains that were resistant to three or less of the drugs tended to dominate the original organism suggesting that under the prevailing growth conditions these strains had a selective growth advantage. Closed culture experiments (Table 2) showed that the four mutant strains carrying plasmids coding for resistance to two or three drugs were capable of faster growth rates with maximum specific growth rates ($\mu_{\max}$) approximately 30 % greater than the parent strain. This trend was continued by the plasmid-minus strain whose $\mu_{\max}$ value was greater than those of the plasmid-containing mutants and twice the $\mu_{\max}$ of *E. coli* K12(TP120). However, these differences may not be

Fig. 4. Competition between *E. coli* K12(TP120) and *E. coli* K12(TP120A) in glucose-limited chemostats at dilution rates of 0.10 h^{-1} (a) and 0.35 h^{-1} (b). Number of viable tetracycline-resistant organisms [*E. coli* K12(TP120)] ($\circ$). Ratio of the number of tetracycline-resistant organisms [*E. coli* K12(TP120)] to tetracycline-sensitive organisms [*E. coli* K12(TP120A)] ($\bullet$).

Table 3. Competition experiments between *E. coli* K12(TP120) and *E. coli* K12(TP120A) at different dilution rates in carbon-limited and phosphorus-limited chemostat cultures

Nutrient limitation	Dilution rate (h^{-1})	Specific growth rate, μ , of uncompetitive population [<i>E. coli</i> K12(TP120)]		Average percentage decrease of μ for uncompetitive population [<i>E. coli</i> K12(TP120)]
		From eqn 1 (Jannasch, 1969)	From eqn 3 (Slater & Bull, 1978)	
Carbon (glucose)	0.10	-0.02 (0)	-0.03 (0)	100
	0.15	0.05	0.05	67
	0.25	0.17	0.17	32
	0.35	0.27	0.26	24
Phosphorus (phosphate)	0.10	0.01	0.02	85
	0.15	0.03	0.04	77
	0.25	0.16	0.16	36
	0.35	0.23	0.21	37

significant during growth under substrate-limited conditions and submaximal specific growth rates. A detailed examination of the kinetics of competition between one pair of organisms differing in sensitivity towards tetracycline, namely *E. coli* K12(TP120) and *E. coli* K12(TP120A), showed that under both carbon-limiting and phosphorus-limiting conditions, and at all the growth rates examined, the tetracycline-sensitive population always had a competitive growth advantage over the tetracycline-resistant population (Fig. 4; Table 3). In these experiments, care was taken to ensure that both the newly inoculated populations were growing exponentially before establishing continuous growth conditions. Thus, differences in the growth dynamics of the competing populations were ascribable to differences in growth parameters and were not obscured by additional factors such as variable lag phases or differences in physiological status. For all competition experiments, the removal of the uncompetitive tetracycline-resistant *E. coli* K12(TP120) followed the expected washout kinetics (Fig. 4). At the highest dilution rate examined ($D = 0.35 \text{ h}^{-1}$) for carbon-limited competition, the percentage decrease in the specific growth rate of the uncompetitive parent population was similar to the difference between the maximum specific growth rates in closed culture (Tables 2 and 3). At the same dilution rate with phosphate as the growth-limiting substrate, the parent strain was even more uncompetitive since, with existing growth conditions determined by *E. coli* K12(TP120A), *E. coli* K12(TP120) was only capable of a growth rate of 0.22 h^{-1} (Table 3). The degree

of competitiveness between the two populations increased with decreasing growth rate of the successful population such that with glucose as the growth-limiting substrate at the lowest dilution rate examined the parent population was essentially washed out as a non-growing population (Table 3).

DISCUSSION

The purpose of this study was to examine the behaviour of *E. coli* containing a plasmid coding for resistance to four drugs under growth conditions where the presence of the resistance mechanisms was unnecessary, that is in an environment which lacked any of the drugs. This situation is relevant to the distribution of drug-resistant organisms throughout the biosphere, particularly away from the selective environments which led to the evolution of resistant organisms, and it is important in assessing the environmental conditions which may influence the persistence of drug-resistant organisms and plasmid stability. In drug-free environments, the plasmid is redundant and environmental pressures could then discriminate against plasmid-containing organisms, since synthesis and replication of the plasmid utilizes elemental and energy resources which otherwise might be diverted to biomass production and a high population growth rate. This principle was demonstrated by Zamenhof & Eichhorn (1967) who showed that amino acid auxotrophs of *Bacillus subtilis* had a marked growth rate advantage over isogenic prototrophic strains under carbon-limited growth conditions. Furthermore, the greater the number of biosynthetic sequences dispensed with by the auxotroph the greater was its growth rate advantage over the prototroph. Conversely organisms which wasted growth nutrients, for example, by overproducing and excreting an amino acid, were at a competitive disadvantage (Zamenhof & Eichhorn, 1967; Baich & Johnson, 1968).

In view of the strongly selective growth conditions occurring in chemostat continuous-flow culture (Cox & Gibson, 1974; Harder *et al.*, 1977; Slater & Bull, 1978) and the fact that there was a large difference between the specific growth rates of plasmid-containing and plasmid-minus strains of *E. coli* (Table 2), it was anticipated that mutant plasmid-minus strains would have been rapidly selected for under nutrient-limited growth conditions. Our experiments showed that this was not the case and, indeed, in only one instance – under phosphate-limited conditions after approximately 700 h growth – was a plasmid-minus population detected. Thus the plasmid of *E. coli* K12(TP120) appeared to be considerably more stable than the *F'**lac* plasmid in *E. coli* under lactose-limited growth conditions (Slater & Bull, 1978). However, unlike the RP1 plasmid in *E. coli* strain W3110, which was stably maintained for up to 144 h growth under conditions of carbon, phosphorus or magnesium limitation at growth rates between 0.05 and 1.0 h⁻¹ (Melling *et al.*, 1977), plasmid TP120 was only partially stable. Particularly under phosphate-limited conditions, the cultures readily lost resistance to one or two drugs which suggested that the plasmid could partially fragment, perhaps producing a smaller plasmid, and hence gain an advantage in growth rate over the parent strain. Large multiple resistance plasmids seem to be formed as the result of the linkage of separately derived resistance genes or groups of genes to a basic replicating and transferring unit (RTF replicon) and this is a reversible process (Cohen, 1976). Clearly, this provides a mechanism for the generation of smaller plasmids by random reassociation without one (or more) of the original DNA segments and preliminary evidence suggests that this was the case here. The fact that tetracycline resistance was regularly lost indicated that this particular sequence was more readily excised from the original plasmid than the other resistance genes. At present we can offer no satisfactory explanation of the observation that the loss of drug resistances occurred more readily under phosphate-limited conditions than under carbon-limited conditions, particularly as the competition experiments between strains K12(TP120) and K12(TP120A) showed a greater growth rate difference under carbon limitation than under phosphorus limitation (Table 3). The loss of the autotransferring capacity in strains

K12(TP120C) and K12(TP120D) also appeared to contribute towards a selective growth advantage either through the loss of the transfer genes or through a mutation resulting in the non-expression of the genes.

The competition studies with *E. coli* strains K12(TP120) and K12(TP120A) showed that putatively smaller plasmids, certainly carrying resistance genes for fewer drugs, in the mutant strain had a considerable growth advantage. Moreover, the lower the concentration of the growth-limiting substrate (that is, at low growth rates) the greater the difference between the mutant and parent growth rate potentials and the greater the advantage to be gained in losing part of the plasmid (Table 3). It is, however, difficult to explain why the removal of part of the plasmid or, indeed, the whole plasmid results in such large increases in the specific growth rate simply in terms of the conservation of limiting growth resources.

Finally, in all the selection experiments, some of which were continued for over 2000 h of continuous growth, very low levels of the uncompetitive plasmid-containing organisms were maintained. This is an observation in agreement with the experiments of Melling *et al.* (1977), although as a percentage of the population our residual resistant populations were several orders of magnitude lower. The reason for the persistence of these populations is not obvious since kinetic theory predicts that in open growth systems uncompetitive populations ought to be completely eliminated. Most probably these low levels are growth system artifacts representing a small population retained as a result, for example, of non-homogeneous conditions within the culture vessel or wall growth. However, it cannot be excluded that some mechanism, so far unidentified, exists whereby low levels of a potentially advantageous capability (drug resistance) are retained within the population even though currently the population may be disadvantaged. Clearly, the long-term survival of the population subjected to future drug challenges would be greatly aided if a few members of the population retained the necessary resistance mechanisms.

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