

Investigation of the Effect of Growth Environment on the Stability of Low-copy-number Plasmids in *Escherichia coli*

By CELIA A. CAULCOTT,^{1*} ANDREW DUNN,^{1†} HEATHER A. ROBERTSON,¹ NEILL S. COOPER,² MICHAEL E. BROWN¹ AND P. MALCOLM RHODES¹

¹Department of Fermentation, Celltech Ltd, 244 Bath Road, Slough, Berks SL1 4DY, UK

²Department of Atmospheric Physics, The University, Oxford, UK

(Received 29 September 1986; revised 22 January 1987)

The stability of a low-copy-number plasmid, pHSG415, in *Escherichia coli*, was investigated in batch and continuous culture. The plasmid was unstable in batch culture, but was significantly stabilized by growth in continuous culture with phosphate, nitrogen or potassium limitation. However, the plasmid was very unstable when grown in continuous culture with sulphate limitation. These results contrast with those obtained with multicopy plasmids such as pBR322, which is particularly unstable in carbon- or phosphate-limited continuous culture. The effect of growth rate on the stability of *E. coli*(pHSG415) grown in continuous culture with glucose limitation was also investigated. The plasmid was significantly more stable in cells grown at higher growth rates. The segregational instability (R) of the plasmid and the difference in growth rate between plasmid-free and plasmid-bearing cells ($d\mu$) were calculated for each condition using the method of Cooper *et al.* (accompanying paper: *Journal of General Microbiology* 133, 1871–1880). It was found that the primary cause of the loss of pHSG415 from the cell population was the segregational instability of the plasmid.

INTRODUCTION

The instability of plasmids in micro-organisms such as *Escherichia coli* can be generally attributed to two overall effects. These are the rate at which the plasmid-bearing cells generate plasmid-free cells, which is described here as the segregational instability of the plasmid (R), and the difference in growth rate ($d\mu$) between the plasmid-free and plasmid-bearing cells (Walmsley *et al.*, 1983; Cooper *et al.*, 1986, 1987). Thus, for plasmids which greatly reduce the growth rate of the host cell, growth-rate difference would be the major factor contributing to the disappearance of plasmid-bearing cells from the population (Caulcott *et al.*, 1985). However, it is not always feasible to determine accurately which, if either, of $d\mu$ and R is the dominant influence in the pattern of instability shown by a plasmid, nor whether it is possible to vary either parameter.

It is known, however, that several factors influence the observed instability of plasmids in micro-organisms. Hence studies of the stability of plasmids such as pAT153 and pBR322 have produced contradictory results, some of which are probably attributable to the use of different host strains (Jones, I. *et al.*, 1980; Jones, S. *et al.*, 1980; Noack *et al.*, 1981; Jones & Melling, 1984; Primrose *et al.*, 1984; Caulcott *et al.*, 1985). The properties of the plasmid itself are also important. Many naturally occurring plasmids, for example, have a complex mechanism for copy-number control (Molin *et al.*, 1981; Som & Tomizawa, 1983) which may include, particularly in low-copy-number plasmids, one or more sequences which assist in the partition of plasmid molecules at cell division (Meacock & Cohen, 1980; Skogman *et al.*, 1983; Primrose

† Present address: Department of Microbiology, University of Wales Institute of Technology, Cardiff, UK.

et al., 1984). Such a mechanism would make the generation of plasmid-free daughter cells less likely. It is interesting to note that cloning vectors tend to lack these stabilizing functions.

It has also been found that modification of the plasmid may affect its stability (Jones & Melling, 1984; Vernet *et al.*, 1985). One particular type of alteration which can lead to considerable instability is the insertion of highly expressed heterologous genes into the plasmid (Imanaka *et al.*, 1980; Miwa & Momose, 1982; Steuber & Bujard, 1982; Skogman *et al.*, 1983; Caulcott *et al.*, 1985).

The growth conditions of the host cells may also affect the stability of plasmids. Glucose, phosphate and magnesium limitation in continuous culture have all been shown to reduce the stability of various plasmids (Godwin & Slater, 1979; Jones, I. *et al.*, 1980; Noack *et al.*, 1981; Jones & Melling, 1984) although no single limitation affected all plasmids tested. Nitrogen limitation was shown to have little effect on the stability of pBR325 (Roth *et al.*, 1980; Wouters *et al.*, 1980; Noack *et al.*, 1981). It has also been found that low growth rates (Melling *et al.*, 1977; Godwin & Slater, 1979; Wouters *et al.*, 1980; results in this paper) and high growth temperatures (Wouters *et al.*, 1980) reduced the stability of various plasmids in *E. coli*. From these results it is clear that those factors which influence plasmid stability are several and varied.

This paper describes the effect of various nutritional limitations and different growth rates on the stability of a low-copy-number plasmid, pSC101, and the derivative, pHSG415. The latter plasmid has a temperature-sensitive origin of replication (Hashimoto-Gotoh *et al.*, 1981) and is partially deleted for stability functions (*par*) (Tucker *et al.*, 1984). The work was done for two significant reasons. It was proposed to use pHSG415 as the basis of a dual-origin cloning vector for the expression of heterologous genes in *E. coli* (Yarranton *et al.*, 1984; Caulcott *et al.*, 1985; Wright *et al.*, 1987). However, because the vector was found to be unstable it was decided to replace the pHSG415 origin of replication and *par* fragment with the entire pSC101 origin of replication and *par* sequence. In addition, initial observations of the instability of *E. coli*(pHSG415) indicated that the loss of plasmid from the host cell followed a different pattern from that observed with other cloning vectors (Caulcott *et al.*, 1985), suggesting that the instability of the plasmid was not due to the growth rate difference it imposed on the host cell, but rather to instability of partitioning of the plasmid at cell division. Hence, in order to investigate this observation the relationship between the growth rate difference and the actual segregational instability of the plasmid pHSG415 in *E. coli* was determined.

METHODS

Bacterial strains and plasmids. The *E. coli* K12 strain RV308 was transformed with either pSC101 or pHSG415 (Hashimoto-Gotoh *et al.*, 1981) and maintained on L-agar supplemented respectively with tetracycline (8 mg l⁻¹) or chloramphenicol (10 mg l⁻¹).

Media. For starter cultures, L-agar supplemented with the appropriate antibiotic and L-broth (supplemented with chloramphenicol for work with pHSG415) were used. For growth in continuous culture the defined salts medium described previously was used (Caulcott *et al.*, 1985), modified as necessary to give the desired nutrient limitation. Antibiotics were not added. Antifoam (polypropylene glycol 2000) was present in the medium at 0.001% (v/v).

Chemostat experiments. For continuous culture experiments, cells were grown in 1 litre LH500 series fermenter vessels (LH Fermentation) at 30 °C. The pH was maintained at 6.5 by the addition of 3 M-NaOH. The fermenters were stirred continuously at 700 r.p.m. and aerated with one volume of air per culture volume per minute. The dissolved oxygen did not fall below 50% saturation. Using the defined salts medium, cells were grown under glucose, nitrogen, phosphate, potassium or sulphate limitation (in each case all other nutrients were in excess) at a dilution rate (*D*) of approximately 0.155 h⁻¹. Cells were also grown under glucose limitation using a variety of dilution rates.

For *E. coli* RV308(pSC101), a single colony was taken from an agar plate containing tetracycline and inoculated into 100 ml L-broth in a 250 ml conical flask with a steel spring baffle. The culture was incubated on an orbital shaker (30 °C, 220 r.p.m.) until stationary phase was reached. The cells were harvested by centrifugation (700 g, 20 °C, 10 min), resuspended in sterile medium minus the desired limiting nutrient and inoculated into a 1 litre fermenter vessel containing defined medium. When the culture reached an optical density at 600 nm of at least 60% of the maximum supported by the medium, the pump was switched on and the system was run as a chemostat.

Table 1. Effect of various concentrations of chloramphenicol on the stability of *E. coli*(pHSG415) grown in batch culture in complex and defined media

Medium in first (inoculum) flask	Chloramphenicol concn (mg ml ⁻¹)	Proportion of plasmid-bearing cells	
		First (inoculum) flask at stationary phase	Second (defined medium) flask at stationary phase*
L-Broth	0	0.71	0.53
	10	ND	0.75
	20	0.71	0.89
Defined medium	0	0.51	0.54
	10	0.75	0.94
	20	0.72	0.88

ND, Not determined.

* Equivalent to initial batch phase in a chemostat culture.

For *E. coli* RV308(pHSG415) a similar procedure was adopted. However, the initial L-agar plate contained chloramphenicol rather than tetracycline. Furthermore, the L-broth starter culture and the medium during the initial batch phase in the fermenter were supplemented with chloramphenicol (20 mg l⁻¹). The feed medium was antibiotic-free.

For all continuous fermentations, once the total biomass in the vessel was constant (generally 24 h or more after switching on the pump), it was assumed that the initial transient growth phase had ceased. From this time the number of generations in the steady state were calculated.

Antibiotic resistance tests. Samples were withdrawn from the continuous cultures, diluted and plated onto L-agar. One hundred single colonies were picked both onto L-agar containing the appropriate antibiotic and L-agar as a control. The number of colonies resistant to the antibiotic was expressed as a percentage of the number growing on the L-agar and was taken to represent the proportion of the population which carried the plasmid.

From the binomial distribution, the error in this method can be determined using the formula

$$\text{standard deviation (SD)} = \sqrt{npq}$$

where $n = 100$ (i.e. the number of colonies picked), p = the number of antibiotic-sensitive colonies and q = the number of antibiotic-resistant colonies. Hence the error on the observed data is greatest when $p = q$ and tends towards zero when either p or q tends towards zero. By use of a statistical analysis of the data, this and other experimental errors are allowed for in the values presented in Results.

RESULTS

Growth of E. coli(pHSG415) and *E. coli*(pSC101) in batch culture

Initially, inocula of *E. coli*(pHSG415) were prepared in the manner described for *E. coli*(pSC101). However, it was found on many occasions that, unlike pSC101, pHSG415 was very unstable in cells grown in batch culture, both in the defined medium normally used for chemostat culture and in L-broth (e.g. data in Table 1). The effect of adding chloramphenicol both to the starter culture medium and the initial batch phase of the chemostat culture was, therefore, investigated. Flasks containing chloramphenicol at 0, 10 or 20 mg l⁻¹ in either L-broth or defined medium were set up and each was inoculated with a single colony of *E. coli*(pHSG415). After growth to stationary phase (which took very much longer for cells growing in defined medium) the proportion of the population carrying the plasmid was determined. Each flask was then used to inoculate a further flask containing defined medium and chloramphenicol at the same concentration as was present in the initial flask. Again, the cells were allowed to grow to stationary phase, and the proportion of the population carrying the plasmid determined.

Since the percentage of antibiotic-resistant cells in an inoculum colony is not known, these data may be subject to some variation. However, the data showed that provided chloramphenicol was present, the choice of medium for the first flask (equivalent to the inoculum for a chemostat) was not important (Table 1). Therefore, since the cells grew faster in L-broth, this medium, containing chloramphenicol at 20 mg l⁻¹, was chosen for the inoculum.

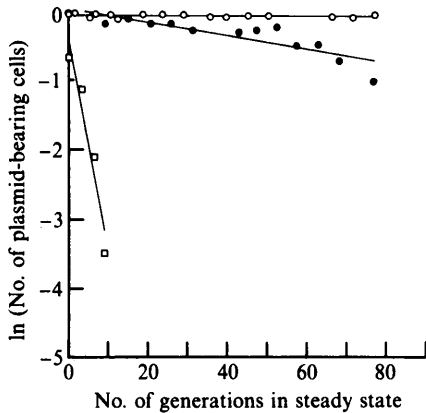


Fig. 1

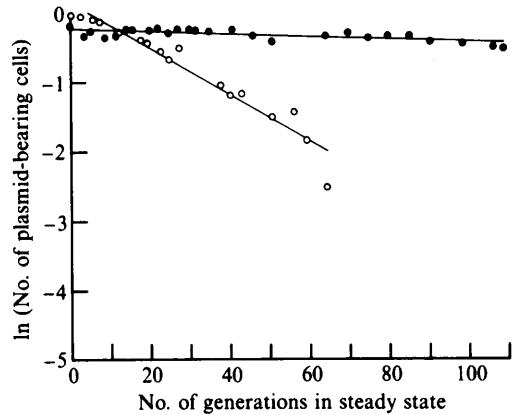


Fig. 2

Fig. 1. Stability of *E. coli*(pHSG415), grown in continuous culture with phosphate, potassium or sulphate limitation at $D = 0.156 \text{ h}^{-1}$. After inoculation into the fermenters the cells were grown initially in batch culture in the presence of chloramphenicol (20 mg l^{-1}). The nutrient flow pump was switched on and, after a transient phase (during which the chloramphenicol was washed out) steady state was achieved. Samples were taken and the proportion of the population expressing the plasmid was determined. ○, Phosphate limitation; ●, potassium limitation; □, sulphate limitation.

Fig. 2. Stability of *E. coli*(pHSG415), grown in continuous culture with nitrogen or glucose limitation at $D = 0.156 \text{ h}^{-1}$. After inoculation into the fermenters the cells were grown initially in batch culture in the presence of chloramphenicol (20 mg l^{-1}). The nutrient flow pump was switched on and, after a transient phase (during which the chloramphenicol was washed out) steady state was achieved. Samples were taken and the proportion of the population expressing the plasmid was determined. ○, Nitrogen limitation; ●, carbon limitation.

Further, in order to establish in the fermenter an initial cell population which contained as many plasmid-bearing cells as possible, chloramphenicol (20 mg l^{-1}) was added to the defined medium for the initial batch phase. Once the medium flow was switched on, residual chloramphenicol would be gradually washed out, ensuring that, when steady state was achieved, the medium was antibiotic-free, and so exerted no overt selective pressure in favour of the plasmid.

To determine whether pSC101 or pHSG415 had an effect on the maximum specific growth rate (μ_{\max}) of the host, *E. coli* RV308, with and without the plasmids, was grown in batch culture in defined and complex media, and μ_{\max} was determined in each case. For a given medium, there was no significant difference between the values of μ_{\max} obtained for the two cell lines (data not shown).

Growth of E. coli(pHSG415) in continuous culture with various nutrient limitations

The stability of *E. coli*(pHSG415) grown in continuous culture with glucose, nitrogen, phosphate, potassium or sulphate limitation was investigated. In all cases the cells were grown at a dilution rate of approximately 0.155 h^{-1} . There was considerable variation in the stability of *E. coli*(pHSG415) grown with different nutrient limitation (Figs 1 and 2). Growing under phosphate limitation, *E. coli*(pHSG415) was very stable, but there was a progressive decrease in the stability of the plasmid with nitrogen, potassium and carbon limitations. When sulphate was the limiting nutrient the plasmid was very unstable indeed, and was lost from the population within 10 generation of entering steady state.

The natural logarithm (ln) of the proportion of plasmid-bearing cells (p_+) was plotted against the number of generations in continuous culture and a linear relationship was observed. Using

Table 2. Values for the segregational instability (R) and growth rate difference ($d\mu$) for *E. coli*(pHSG415) grown in continuous culture at a constant dilution rate (0.155 h^{-1}) with different nutrient limitations

Limitation	R		$d\mu$	
	(gen^{-1})	95% confidence limits	(gen^{-1})	95% confidence limits
Phosphate	2.1×10^{-4}	0 to 8.0×10^{-4}	-0.13×10^{-5}	-1.0×10^{-5} to 1.0×10^{-5}
Nitrogen	2.0×10^{-3}	1.3×10^{-3} to 2.8×10^{-3}	-6.1×10^{-5}	-12.0×10^{-5} to -2.0×10^{-5}
Potassium	9.2×10^{-3}	5.4×10^{-3} to 13.6×10^{-3}	6.3×10^{-4}	-0.7×10^{-4} to 19.9×10^{-4}
Carbon	2.8×10^{-2}	2.3×10^{-2} to 3.4×10^{-2}	5.3×10^{-3}	2.8×10^{-3} to 8.6×10^{-3}
Sulphate	0.26	0 to 0.99	0.044	-0.4 to 4.3

these plots, it was possible to calculate the segregational instability (R) of the plasmid and any growth-rate difference ($d\mu$) which there might be between the plasmid-bearing and the plasmid-free cells. The equations used were

$$R = \frac{-m}{(e^c + p_{-0})}$$

$$d\mu = \frac{(1 - e^c - p_{-0})}{(e^c + p_{-0})}$$

(Cooper *et al.*, 1986)

where m = slope of the line [from the graph of natural logarithm (plasmid-bearing cells) against time], c = intercept, p_{-0} = proportion of plasmid-free cells at time $t=0$ and t = time in generations.

The results of these calculations are shown in Table 2. In all cases, the segregational instability of the plasmid was one to two orders of magnitude greater than the growth rate difference. This indicates that the primary cause of loss of pHSG415 from *E. coli* RV308 when grown in continuous culture was the segregational instability of the plasmid. The rate of loss was particularly high under carbon and sulphate limitations; with the latter a rate of 0.259 gen^{-1} represents the loss of the plasmid from a quarter of the cell population every cell division.

When cells were grown under conditions of phosphate or nitrogen limitation, $d\mu$ was negligible. However, with the remaining three limitations there was a significant growth rate difference; accordingly, overgrowth of the plasmid-bearing population by the plasmid-free cells became a real possibility. Nevertheless, the higher growth rate of the plasmid-free cells did not contribute significantly to the loss of the plasmid from the population, even in the sulphate-limited culture, since the segregational instability of the plasmid was an order of magnitude greater in every case.

Growth of E. coli(pHSG415) with carbon limitation at various dilution rates

The stability of *E. coli*(pHSG415) when grown in continuous culture with carbon limitation at dilution rates (D) of 0.156 , 0.304 , 0.404 and 0.495 h^{-1} was investigated (Fig. 3). It was found that the plasmid was considerably more stable at the higher dilution rates (0.404 and 0.495 h^{-1}) than at the lower dilution rates. It also appeared that the plasmid might be slightly less stable when the host was grown at $D = 0.304 \text{ h}^{-1}$ than at $D = 0.156 \text{ h}^{-1}$.

The values of R and $d\mu$ were calculated (Table 3). At the high dilution rates the values for $d\mu$ were negligible in relation to R , indicating that the very limited loss of plasmid observed was due to the segregational instability of the plasmid. Interestingly, there was very little difference between the values of R determined for the populations grown at $D = 0.404 \text{ h}^{-1}$ and 0.495 h^{-1} . It is possible that very similar kinetics of loss would be observed for any culture of *E. coli*(pHSG415) grown under conditions of carbon limitation at dilution rates greater than 0.4 h^{-1} .

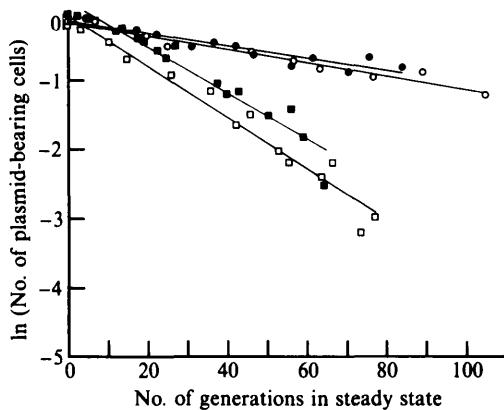


Fig. 3. Stability of *E. coli*(pHSG415), grown in continuous culture with glucose limitation at various dilution rates. After inoculation into the fermenters the cells were grown initially in batch culture in the presence of chloramphenicol (20 mg l^{-1}). The nutrient flow pump was switched on and, after a transient phase (during which the chloramphenicol was washed out) steady state was achieved. Samples were taken and the proportion of the population expressing the plasmid was determined. \circ , $D = 0.495 \text{ h}^{-1}$; \bullet , $D = 0.404 \text{ h}^{-1}$; \square , $D = 0.304 \text{ h}^{-1}$; \blacksquare , $D = 0.156 \text{ h}^{-1}$.

Table 3. Values for the segregational instability (R) and growth rate difference ($d\mu$) for *E. coli*(pHSG415) grown in continuous culture with carbon limitation at various dilution rates

Dilution rate (h^{-1})	R (gen^{-1})	95% confidence limits	$d\mu$ (gen^{-1})	95% confidence limits
0.495	9.8×10^{-3}	7.8×10^{-3} to 12.0×10^{-3}	-1.7×10^{-4}	-7×10^{-4} to 34×10^{-4}
0.404	8.8×10^{-3}	6.5×10^{-3} to 11.4×10^{-3}	3.4×10^{-5}	-5×10^{-4} to 6×10^{-4}
0.304	3.3×10^{-2}	2.7×10^{-2} to 3.9×10^{-2}	3.8×10^{-3}	1.3×10^{-3} to 7.4×10^{-3}
0.156	2.8×10^{-2}	2.3×10^{-2} to 3.4×10^{-2}	5.3×10^{-3}	2.8×10^{-3} to 8.6×10^{-3}

The values obtained for R and $d\mu$ for cells grown at $D = 0.304 \text{ h}^{-1}$ and 0.156 h^{-1} were also similar. Although both parameters were slightly greater at $D = 0.304 \text{ h}^{-1}$ than at $D = 0.156 \text{ h}^{-1}$, there was no significant difference between them at the 95% probability level. As with other nutrient limitations, the data support the conclusion that the primary cause of the loss of pHSG415 from the culture was segregational instability, and not growth rate advantage exhibited by the plasmid-free population.

Stability of *E. coli*(pSC101) in continuous culture

The stability of *E. coli*(pSC101) in continuous culture with nitrogen, phosphate, carbon, sulphate and potassium limitation, at growth rates of $D = 0.155 \text{ h}^{-1}$, and with carbon limitation, at $D = 0.500 \text{ h}^{-1}$, was investigated. The plasmid was found to be 100% stable under all conditions examined (data not shown).

DISCUSSION

The data obtained indicate that *E. coli*(pHSG415) was unstable in the batch cultures described here, and when grown with certain nutrient limitations in continuous culture. This finding is in contrast both to the parent plasmid, pSC101, which was stable under all growth conditions investigated, and to reports of Hashimoto-Gotoh *et al.* (1981) that *E. coli*(pHSG415) is stable in batch culture in complex medium through 100 generations. This latter result may possibly be attributable to host strain differences (Summers & Sherratt, 1984) or to variation in

Table 4. Probability of generation of plasmid-free cells from plasmid-bearing cells due to random segregation of plasmids of different copy-number

Copy-number (n)	Copy-number at cell division ($2n$)	P_0 † (gen ⁻¹)
1	2	0.5
2	4	0.125
3	6	0.031
4	8	7.81×10^{-3}
5	10	1.95×10^{-3}
6	12	4.88×10^{-4}
10	20	1.91×10^{-6}

† P_0 is calculated from binominal probability theory according to the formula $P_0 = 2(1/2)^{2n}$ (Meacock & Cohen, 1980). $P_0 = R$ when there is no plasmid- or host-mediated stability function.

the chosen culture conditions. The difference between pSC101 and pHSG415 is probably due to the absence of most of the *par* sequence from pHSG415. Since both pSC101 and pHSG415 are low-copy-number plasmids (approximately five copies per host chromosome), they have a relatively high probability of being lost at cell division unless actively partitioned (Table 4). The *par* sequence present in pSC101 is believed to ensure that both daughter cells contain at least one copy of the plasmid after cell division (Meacock & Cohen, 1980; Skogman *et al.*, 1983), thereby greatly reducing the probability that plasmid-free daughter cells would arise. A reduction in the efficiency of this system would be expected to cause an increase in the segregational instability of a plasmid, as was observed with *E. coli* RV308(pHSG415) when grown in continuous culture under some nutrient limitations. Similar results were also observed by Tucker *et al.* (1984) on deletion of various segments of the *par* region of pSC101-derived plasmids.

Despite the reduced *par* sequence, the segregation of pHSG415 at cell division still may not be an entirely random process. If it were, no variation in the kinetics of plasmid loss would be expected when the cells were grown under a variety of culture conditions, including different growth rates. The results presented here demonstrate considerable variation in the segregational instability of pHSG415, and, therefore, in the kinetics of loss of the plasmid. In particular, the plasmid appeared to be more stable than would be predicted when the cells were grown with phosphate, nitrogen or potassium limitations. In contrast, the rate of loss of the plasmid from cells grown with sulphate limitation was very much higher than might have been expected.

It is possible that an explanation for these observations is the effect of the temperature-sensitive origin of replication of pHSG415. However, Hashimoto-Gotoh *et al.* (1981) argued that the origin of replication functions correctly at 30 °C, by demonstrating that the plasmid is stable for over 100 generations in their growth condition. Nevertheless, it would be valuable to examine the stability of a *rep-ts par*⁺ derivative of pSC101.

For many of the *E. coli* plasmids previously investigated in continuous culture, carbon and phosphate limitations have given rise to the greatest degree of plasmid instability (Wouters *et al.*, 1980, Noack *et al.*, 1981; Jones & Melling, 1984). Many of the plasmids studied have been either multicopy vectors or large, resistance-factor plasmids such as RP1 (Melling *et al.*, 1977). It may be significant, therefore, that pHSG415, a small, low-copy-number plasmid, is stable when grown under conditions of phosphate limitation. It is possible that there is a relationship between the nutritional conditions in which a host-plasmid system is grown and the copy-number of the plasmid, as observed by Engberg & Nordstrom (1975) and Seigel & Ryu (1985), who found that plasmid copy-number was inversely proportional to host cell growth rate. An alternative explanation is that the effect which some plasmids exert on the metabolism of their hosts is significant. It is not known whether the instability of plasmids such as pBR322 is caused by segregational instability or growth rate difference, but it is possible that plasmids which impose a metabolic load on their host cell ($d\mu > R$) are subject to certain limitations which lead to high rates of plasmid loss, whereas in the case of plasmids where $R > d\mu$, such as pHSG415, there could be an alternative group of nutritional limitations which cause the plasmids to be very unstable.

Although there are variations in the effect different nutritional limitations have on the stability of plasmids, more consistent results have been found with the effect of growth rate since, in general, it has been found that plasmids are more stable at higher growth rates (Melling *et al.*, 1977; Godwin & Slater 1979; Roth *et al.*, 1980; Wouters *et al.*, 1980). Similar results were observed in the experiments with *E. coli* RV308(pHSG415), although an orderly relationship between growth rate and plasmid instability was not found.

Perhaps the most interesting aspect of all these results is that associated with the variation in the stability of *E. coli*(pHSG415) with different growth conditions. In particular, having observed that pHSG415 was unstable in *E. coli* RV308 grown in batch culture, it was very surprising to find that the same host and plasmid combination was essentially stable when grown in continuous culture with phosphate or nitrogen limitation. In general, previous workers investigated the instability of plasmids found to be stable in batch culture but unstable in continuous culture (Wouters *et al.*, 1980, Noack *et al.*, 1981; Jones & Melling, 1984). The results here may be specific to *E. coli* RV308(pHSG415) but they do lead to interesting questions. For example, are there physiological ways to improve the stability of unstable plasmids? Is there a relationship between the growth environment and the effect a plasmid exerts on its host? The answers to questions such as these will undoubtedly contribute significantly to the understanding of how bacteria interact both with their plasmids and their environment.

The authors wish to thank Dr Geoff Yarranton for valuable advice and discussion and Lynn Dowley for secretarial assistance.

REFERENCES

- CAULCOTT, C. A., LILLEY, G., WRIGHT, E. M., ROBINSON, M. K. & YARRANTON, G. T. (1985). Investigation of the instability of plasmids directing the expression of Met-prochymosin in *Escherichia coli*. *Journal of General Microbiology* **131**, 3355-3365.
- COOPER, N. S., BROWN, M. E., RHODES, P. M. & CAULCOTT, C. A. (1986). A method for investigating factors affecting the instability of plasmids in micro-organisms. *Society for General Microbiology 106th Meeting Abstracts*, p. 47.
- COOPER, N. S., BROWN, M. E. & CAULCOTT, C. A. (1987). A mathematical method for analysing plasmid stability in micro-organisms. *Journal of General Microbiology* **133**, 1871-1880.
- ENGBERG, B. & NORDSTROM, K. (1985). Replication of R-factor R1 in *Escherichia coli* K-12 at different growth rates. *Journal of Bacteriology* **123**, 179-186.
- GOODWIN, D. & SLATER, J. H. (1979). The influence of the growth environment on the stability of a drug resistance plasmid in *Escherichia coli* K12. *Journal of General Microbiology* **111**, 201-210.
- HASHIMOTO-GOTOH, T., FRANKLIN, F. C. H., NORDHEIM, A. & TIMMIS, K. N. (1981). Specific-purpose plasmid cloning vectors. 1. Low-copy number, temperature sensitive, mobilisation-defective pSC101-derived containment vectors. *Gene* **16**, 227-235.
- IMANAKA, T., TSUNEKAWA, H. & AIBA, S. (1980). Phenotypic stability of *trp* operon recombinant plasmids in *Escherichia coli*. *Journal of General Microbiology* **118**, 253-261.
- JONES, I. M., PRIMROSE, S. B., ROBINSON, A. & ELLWOOD, D. C. (1980). Maintenance of some ColE1-type plasmids in chemostat culture. *Molecular and General Genetics* **180**, 579-584.
- JONES, S. A. & MELLING, J. (1984). Persistence of pBR322-related plasmids in *Escherichia coli* grown in chemostat culture. *FEMS Microbiology Letters* **22**, 239-243.
- JONES, S. A., DEARNLEY, K., BENNETT, P. M. & MELLING, J. (1980). The stability of antibiotic resistance plasmids in *Escherichia coli* hosts grown in continuous culture. *Society for General Microbiology Quarterly* **8**, 44.
- MEACOCK, P. A. & COHEN, S. N. (1980). Partitioning of bacterial plasmids during cell division: a *cis*-acting locus that accomplishes stable plasmid inheritance. *Cell* **20**, 529-542.
- MELLING, J., ELLWOOD, D. C. & ROBINSON, A. (1977). Survival of R-factor carrying *Escherichia coli* in mixed cultures in the chemostat. *FEMS Microbiology Letters* **2**, 87-89.
- MIWA, K. & MOMOSE, H. (1982). Micro-organisms harbouring a plasmid, with stabilised characteristics, and a method of obtaining it. European Patent Application no. 0019877-A1.
- MOLIN, S., STOUGAARD, P., LIGHT, J., NORDSTROM M. & NORDSTROM, K. (1981). Isolation and characterisation of new copy number mutants of plasmid R1 and identification of a polypeptide involved in copy number control. *Molecular and General Genetics* **181**, 123-130.
- NOACK, D., ROTH, M., GEUTHER, R., MULLER, G. & UNDISZ, K. (1981). Maintenance and genetic stability of vector plasmids pBR322 and pBR325 in *Escherichia coli* K12 strains grown in a chemostat. *Molecular and General Genetics* **184**, 121-124.
- PRIMROSE, S. B., DERBYSHIRE, P., JONES, I. M., ROBINSON, A. & ELLWOOD, D. C. (1984). The application of continuous culture to the study of plasmid stability. In *Continuous Culture* **8**, pp. 213-238. Edited by A. C. R. Dean, D. C. Ellwood & C. G. T. Evans. Chichester: Ellis Horwood.
- ROTH, M., MULLER, G. & NOACK, D. (1980). Loss of the multicopy resistance plasmid pBR325 from *Escherichia coli* GY2354 pBR325 during continuous cultivation. In *Antibiotic Resistance*, p. 143. Edited by S. Mitsuhashi, L. Rosnal & V. Kremery. Prague: Avicenuma/Springer-Verlag.

- SIEGUL, R. & RYU, D. D. Y. (1985). Kinetic study of instability of recombinant plasmid pPLc23 *trpAI* in *E. coli* using two-stage continuous culture system. *Biotechnology and Bioengineering* **27**, 28–33.
- SKOGMAN, G., NILSSON, J. & GUSTAFSSON, P. (1983). The use of a partition locus to increase stability of tryptophan-operon-bearing plasmids in *Escherichia coli*. *Gene* **23**, 105–115.
- SOM, T. & TOMIZAWA, J. U. (1983). Regulatory regions of ColE1 that are involved in determination of plasmid copy number. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 3282–3286.
- STEBER, D. & BUJARD, H. (1982). Transcription from efficient promoters can interfere with plasmid replication and diminish expression of plasmid specific gene. *EMBO Journal* **11**, 1399–1404.
- SUMMERS, D. K. & SHERRATT, D. J. (1984). Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. *Cell* **36**, 1097–1103.
- TUCKER, W. T., MILLER, C. A. & COHEN, S. N. (1984). Structural and functional analysis of the *par* region of the pSC101 plasmid. *Cell* **38**, 191–201.
- VERNET, T., McDONALD, I. J., CAMERON, D. R. & VISENTIN, L. P. (1985). Stable maintenance in chemostat-grown *Escherichia coli* of pBR322 and pACYC184 by disruption of the tetracycline resistance gene. *Bioscience Reports* **5**, 29–37.
- WALMSLEY, R. M., GARDNER, D. C. J. & OLIVER, S. G. (1983). Stability of a cloned gene in yeast grown in chemostat culture. *Molecular and General Genetics* **192**, 361–365.
- WOUTERS, J. T. M., DRIEHUIS, F. L., POLACZEK, P. J., VAN OPPERNRAAY, M. L. H. A. & VAN ANDEL, J. G. (1980). Persistence of the pBR322 plasmid in *Escherichia coli* K12 grown in chemostat cultures. *Antonie van Leeuwenhoek* **46**, 353–362.
- WRIGHT, E. M., HUMPHRIES, G. O. & YARRANTON, G. T. (1987). Dual origin plasmids containing an amplifiable ColE1 origin; use in the expression of cloned genes. *Gene* **49**, 311–321.
- YARRANTON, G. T., WRIGHT, E., ROBINSON, M. K. & HUMPHREYS, G. O. (1984). Dual-origin plasmid vectors whose origin of replication is controlled by the coliphage lambda promoter P_L . *Gene* **28**, 293–300.