Storage Death at Low Temperature (−18 °C) of Strains of *Escherichia coli* with Different Repair Capacities

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INTRODUCTION

Micro-organisms can only grow within a certain range of temperatures, this range being different for different groups of organisms. Outside these respective temperature ranges, not only does growth stop but, depending on the temperature and composition of the suspending medium, the micro-organisms are injured or inactivated (cf. Ingram & Mackey, 1976; Tomlins & Ordal, 1976). Thus, raising the temperature above the tolerated range offers a convenient and well-known means for reducing the viability of contaminating micro-organisms (sterilization). Reducing the temperature can aid in preserving micro-organisms on storage. The extent to which they will survive such storage in the cold depends on the temperature, the storage time, and the methods used for decreasing the temperature and, later, for raising it again to the growth temperature. The dependence on these and other parameters is complicated and not well-understood (cf. Ingram & Mackey, 1976; Macleod & Calcott, 1976).

The study reported here is concerned with storage death of *Escherichia coli* at −18 °C. The work was initiated through a desire to store bacteria (for experiments on mutation induction) at temperatures as low as possible but in a suspending medium which would not freeze. It was found that bacteria lose their colony-forming ability on prolonged storage, that the death rate may be different for different strains and that this does not depend on the bacterium's repair capability.

METHODS

The bacterial strains are listed in Table 1. The growth medium was LB medium [1 % (w/v) Bacto tryptone, 0·5 % (w/v) Bacto yeast extract, 1 % (w/v) NaCl] and was solidified, where necessary, with 1·6 % (w/v) agar (Difco). Liquid cultures were grown to stationary phase at 30 or 37 °C. Each culture was then divided into two. One half was centrifuged and washed twice with buffered saline [0·7 % (w/v) $Na_2HPO_4.7H_2O$, 0·3 % (w/v) KH_2PO_4, 0·4 % (w/v) NaCl; pH 7·0], and finally resuspended in the original volume of buffered saline. To both this suspension and the other half of the culture, glycerol was added to give 50 % (v/v), to prevent freezing. From these suspensions, 0·3 ml portions were transferred to unsealed 2 ml ampoules and placed at the bottom of a freezer chest at −18 °C. Survival was measured by placing ampoules in a 20 °C water bath, and then plating appropriate dilutions by the agar layer method (bottom layer, 25 ml solidified LB medium; top layer, 2·5 ml containing 0·9 % NaCl and 0·7 % agar; a third layer of this soft agar was added to ensure that all colonies would be within the agar). Colonies were counted after 2 d at 37 °C.

RESULTS

Results of survival experiments with strains of *E. coli* K12 and *E. coli* 15 grown at 37 °C are shown in Fig. 1 (panels A1 to F2). In similar experiments done with 18 strains of *E. coli* B and derivatives of *E. coli* B differing in their growth requirements and repair capacities, survival on the first day of measurement (i.e. the tenth day of storage) was already below 10^{-3} (results not shown).

For some experiments on mutation induction, it was intended to include temperature-

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Table 1. *Strains of Escherichia coli*

Strain no.	Relevant marker	Source	Panel of Fig. 1
W3110	$polA^{+}$ $(\lambda)^{-}$	J. Cairns	A1
W3110 (λ)	$polA^{+}$ $(\lambda)^{+}$	R. Thomas	A2
W3110	*pol* p3478	J. Cairns	A3
W3110	$polA_{am}$	J. Cairns	A4
AB1157	uvr^{+} rec^{+}	R. Devoret	B1
AB1886	*uvrA*	R. Devoret	B2
AB2463	*recA*	R. Devoret	B3
W3623	uvr^{+}	J. Tomizawa	C1, G1
N17-9	*uvrA*	J. Tomizawa	C2
N3-5	*uvrB*	J. Tomizawa	C3
N17-7	*uvrC*	J. Tomizawa	C4
N14-4	*uvrD*	J. Tomizawa	C5
JC4583	uvr^{+} rec^{+}	S. D. Barbour	D1
JC4584	*uvrB recC*	S. D. Barbour	D2
JC4588	*recA*	S. D. Barbour	D3
SDB1006	*recA recB recC*	S. D. Barbour	D4
15 TAU-bar		P. C. Hanawalt	E
DM800	lex^{+}	D. Mount	F1
DM803	*lexA*	D. Mount	F2
B/r	lon^{+} uvr^{+} lex^{+}	A. Novick	G2
B	*lon*	R. Hill	G3
B_{s-1}	*uvrB lexA*	R. Hill	G4

sensitive mutants of some of the strains in Table 1. We therefore examined whether survival of the parent strains of these mutants was influenced by the growth temperature prior to storage. For both suspending media, there was no difference in survival between cultures of a given strain grown at 30 °C and those grown at 37 °C (results not shown). However, in this experiment the sensitivities of the strains were different from those shown in Fig. 1 (panels A1 to F2).

Since the growth temperature did not appear to affect survival and because of the differences in sensitivity between the above two experiments, the survival experiments of Fig. 1 were repeated with all cultures grown at 30 °C; all strains were found to be more resistant. Results are included in Fig. 1 for one of the *E. coli* K12 strains (panel G1, cf. panel C1) and for some strains of the *E. coli* B family (panels G2 to G4).

Repetition of these experiments with some selected strains revealed fluctuations in apparent sensitivities between those of the first and last experiments.

DISCUSSION

The results show that bacterial strains of the same species can differ markedly in their sensitivity to storage at low temperatures under the experimental conditions tested. However, this sensitivity is not influenced by any of the known repair mechanisms investigated. In other words, the inactivating lesions during cooling, storage or warming cannot be repaired to any detectable extent by any of these repair mechanisms.

Despite the variability for some of the strains either within the same experiment or from one experiment to the other, it can be concluded that sensitivity is genetically controlled. This is particularly apparent from comparison of panels D1 and D2 with D3 and D4 of Fig. 1. The difference probably reflects the different origins and methods of construction of these strains (Barbour & Clark, 1970; Capaldo-Kimball & Barbour, 1971; Capaldo *et al.*, 1974). The lower sensitivity seen in D3 and D4 is probably not due to the introduction of the Rec deficiency since at least one other *rec* strain is as sensitive as its parent (compare panels B1 and B3), although it could possibly be due to the particular *rec* allele.

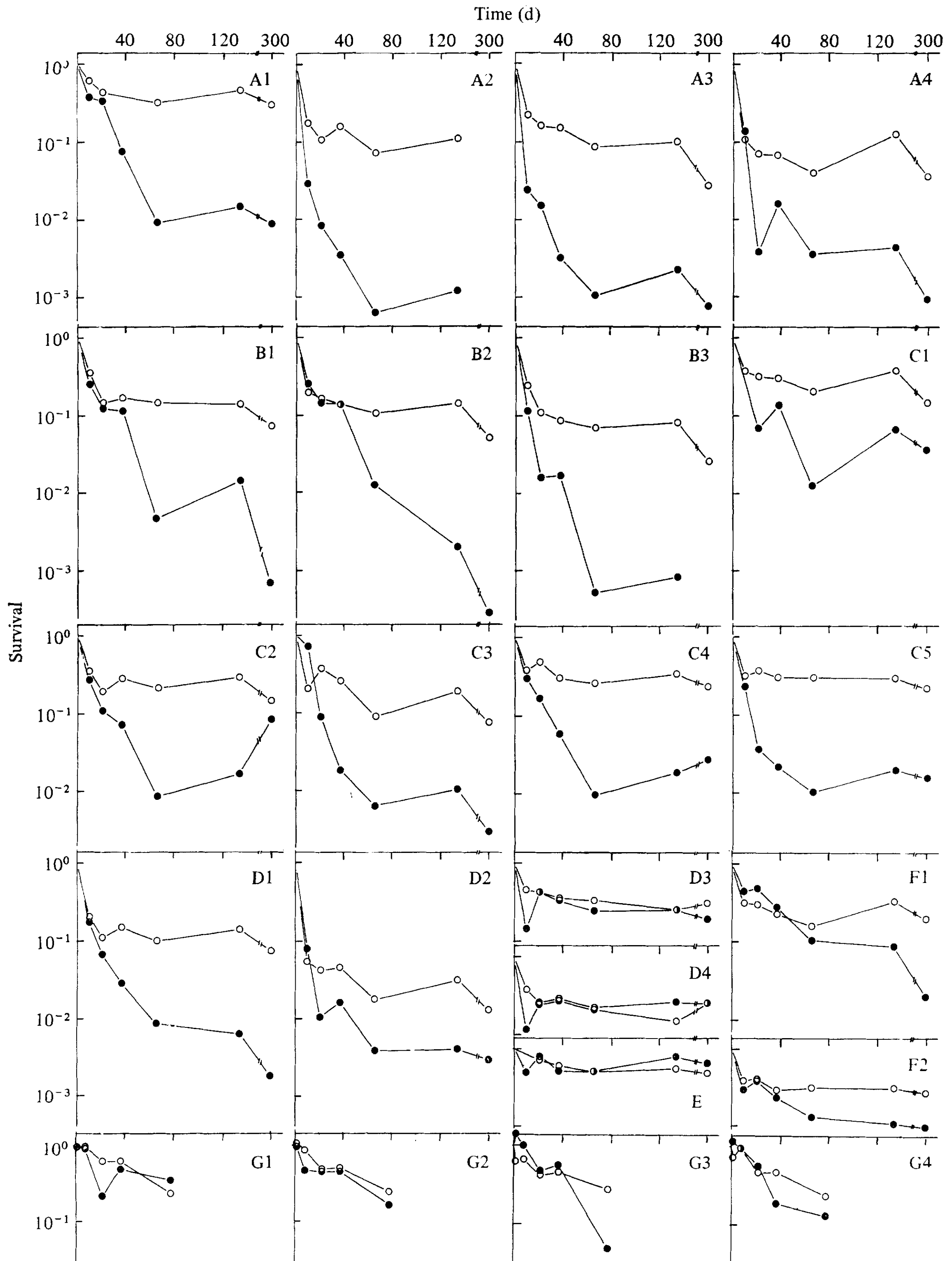

Fig. 1. Survival of *Escherichia coli* strains with different repair capacities after storage at −18 °C in media containing 50 % (v/v) glycerol. Overnight cultures grown at 37 °C (A1 to F2) or 30 °C (G1 to G4) in LB medium were stored at −18 °C in LB medium (●) or in buffered saline (○), both with 50 % (v/v) glycerol. The key to the panels is given in Table 1.

That sensitivity is genetically controlled is also consistent with other observations: (i) whatever the apparent sensitivity of other strains, strain 15 TAU-bar never lost its colony-forming ability beyond that shown in panel E; (ii) in the first experiment, all 18 strains of the *E. coli* B family were considerably more sensitive than the other strains tested; (iii) however pronounced the difference in sensitivity of bacteria stored in the two suspending media was for other strains, this difference was always very small for strains DM800 and DM803 (panels F1 and F2).

The experiments reported here are preliminary, but they allow some conclusions to be drawn. (1) The magnitude of storage death at −18 °C under our experimental conditions is genetically controlled by one or more genes which differ from those responsible for other known repair mechanisms. (2) Although storage death can depend on the growth rate (cf. MacLeod & Calcott, 1976), no differences were detected between cultures grown at 30 °C and those grown at 37 °C. (3) Storage death depends on the composition of the suspending medium (cf. Ingram & Mackey, 1976; MacLeod & Calcott, 1976), survival being better in buffered saline than in broth. (4) Depending on the procedure, storage death can be slow; but for it to be reproducible, experimental conditions have to be controlled precisely. Since for any one experiment the curves were comparatively smooth compared with the gross differences between experiments, it must be concluded that under our experimental conditions cooling was the least controllable step. Thus, it seems that cold storage is impracticable for laboratories not specially equipped to carry out the whole procedure under reproducible conditions.

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