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Modified Penicillin Enrichment Procedure for the Selection of Bacterial Mutants

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Penicillin enrichment and selection of biochemical mutants was performed on agar plates. With this technique, there is an optimum penicillin exposure time for the greatest yield of a particular auxotroph.

The classical technique for the enrichment of biochemical mutants (5, 9) takes advantage of the fact that penicillin can kill only growing cells (4) by inhibiting the cross-linking of peptidoglycan polymers essential for the structural integrity of the cell wall (8, 12). Various modifications have been proposed to circumvent certain problems inherent in the original method (2, 7, 10).

This report describes a modification of the penicillin enrichment technique in which the primary consideration is the duration of the penicillin treatment. This modified technique has the advantage of requiring a minimal number of manipulations, since the penicillin treatment and the selection of the auxotrophs are performed on the same agar plate.

Escherichia coli NP2 (3) was mutagenized with nitrosoguanidine according to the procedure described by Adelberg et al. (1). After mutagenesis the cells were washed once and diluted 10-fold into minimal-glucose medium (3) supplemented with 200 μg/ml of the amino acid necessary to satisfy the nutritional requirement of the desired auxotroph. The sample was incubated overnight with shaking at 37 C. The overnight culture was washed once and diluted 20-fold into minimal glucose medium. The culture was incubated with shaking at 37 C for 1.5 h during which time the viable cell count increased from 10^8 cells/ml to 4×10^8 cells/ml i.e., two divisions. The culture was serially diluted and plated onto minimal glucose agar plates containing 2,000 U of penicillin G (Squibb) per ml of agar and the plates incubated at 37 C for various lengths of time.

The penicillin was inactivated by spreading penicillinase (Sigma Chemical Co.) onto the plates using a sterile bent glass rod to give a final concentration of 4,000 U of penicillinase per ml of agar. The plates were left at room temperature for 1 h. The necessary amino acid required by the desired auxotroph was spread onto the agar plates to give a final concentration of 100 μg/ml of agar and the plates were reincubated at 37 C for 48 h. Those colonies which appeared were picked and tested for the desired auxotrophic requirement.

Figure 1 shows the rate at which penicillin kills wild-type *E. coli* cells using the method described in this report. The ideal recovery efficiency (mutants/survivors) of a biochemical mutant can be calculated for any penicillin exposure time based on the results shown in Fig. 1 by assuming that 0.1% of the mutagenized cells are of any particular mutant type (1); and that during the starvation period before the penicillin exposure a biochemical mutant will undergo one doubling (11) while the wild-type cells undergo two doublings. Thus, before the penicillin exposure, 0.05% of the total cell population would be of the selected mutant type. If there is no loss of mutants during the penicillin treatment, then the ideal recovery efficiency is equal to the number of mutants (0.05% of the cells plated) divided by the total number of survivors, i.e., the percentage of wild-type cells surviving (Fig. 1) plus 0.05% mutants. In practice, however, some of the biochemical mutants will be lost due to cross-feeding, whereby a nongrowing mutant can resume growth by utilizing metabolites released when the nonselected cells are lysed by the action of penicillin. The actual recovery efficiencies will therefore be equal to or less than the ideal efficiencies and will depend on the cross-feeding ability of the particular auxotroph. Comparing the actual recovery efficiencies of three mutants with the ideal efficiencies (Table 1), it can be seen that the longer the penicillin treatment the greater the deviation from the ideal recovery efficiency, presumably due to cross-feeding; furthermore the optimum

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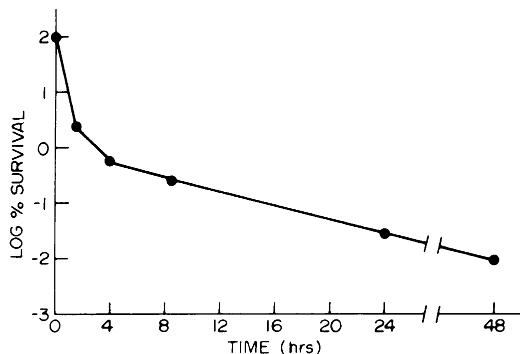

FIG. 1. *The penicillin inactivation rate for wild-type E. coli NP2. An overnight culture of wild-type cells was diluted 100-fold into fresh minimal-glucose medium and incubated at 37 C until logarithmic phase (4 × 10^8 cells/ml). Serial dilutions of these cells were then plated onto the surface of minimal-glucose agar plates containing 2,000 U of penicillin per ml of agar. The plates were incubated at 37 C, and at various time intervals were removed and treated with penicillinase as described in the text. The plates were reincubated at 37 C for 48 h, and the number of survivors was then determined. The percentage of survival is equal to number of survivors/number of cells plated × 100.*

TABLE 1. *Recovery efficiency of biochemical mutants as a function of the penicillin exposure time*

Penicillin exposure time (h)	Recovery efficiency[a]			
	Ideal[b]	Arginine	Histidine	Isoleucine
4	8	10	8	7
24	65	1	45	0
48	90	0	12	0

[a] Recovery efficiency is equal to the number of mutants divided by the total number of survivors ×100.

[b] Ideal recovery efficiency is the recovery efficiency calculated under the ideal condition of no cross-feeding (see text).

exposure time was dependent on the particular auxotroph.

An adaptation of the penicillin selection technique described above has also been used for the isolation of temperature-sensitive mutants defective in the synthesis of certain macromolecules, e.g., proteins (6). After mutagenesis the cells were grown overnight in enriched medium at 30 C. The culture was then diluted 100-fold into fresh broth and incubated at 30 C until early logarithmic phase. The sample was placed at 42 C for 20 min and appropriate dilutions were plated onto nutrient agar plates (prewarmed at 42 C) containing 2,000 U of penicillin per ml of agar. The plates were incubated at 42 C for 24 h and then treated with penicillinase as described previously. The plates were reincubated at 42 C for 24 h and any colonies which appeared were marked on the bottom of the petri dish. The plates were incubated for 36 h at 30 C and any new colonies which appeared were tested for temperature sensitivity. Between 30 and 40% of the colonies were found to be temperature sensitive.

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