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Selection of Thymine-requiring Strains from *Escherichia coli* on Solid Medium

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Aminopterin has been used as a selective agent for thymine-requiring mutants for some time (T. Okada, K. Yanagisawa, and F. J. Ryan, Z. Vererbungslehre **92**:403, 1961; T. Okada, J. Homma, and H. Sonohara, J. Bacteriol. **84**:602, 1962). This method involving selection in liquid cultures has been used successfully in this laboratory. However, some *Escherichia coli* strains grow only very slowly in the unsupplemented liquid medium. Also, the selection sometimes fails, owing to the overgrowth by bacteria which do not require thymine on subsequent testing. These may be unstable thymine auxotrophs which revert quickly in the absence of aminopterin. In addition, a separate selection must be performed for each independent mutant desired. I have developed a variation on the usual technique which overcomes these difficulties and permits the selection of a large number of *thy*$^-$ mutants from *E. coli* strains. The main feature of the procedure is the incorporation of aminopterin into a solid enriched medium.

A culture of the strain to be made *thy*$^-$ is grown overnight with aeration in a defined liquid medium lacking thymine. About 10^8 cells (in 0.1 ml) are then spread on a plate containing supplemented A medium (in grams per liter): K_2HPO_4, 10.5; KH_2PO_4, 4.5; Na citrate·$5H_2O$, 0.47; $MgSO_4$, 0.05; $(NH_4)_2SO_4$, 1.0; agar, 15; Casamino Acids, 5; adenosine, guanosine, cytidine, thymidine, and tryptophan, each 0.1; aminopterin, 0.5; glucose, 2; and thiamine, 0.001. The plate is incubated about 24 hr at 37 C. Some strains require longer incubation. Scattered across a confluent background of bacterial growth are a number of colonies of aminopterin-resistant bacteria, most of which are thymine-requiring. Since the culture which was spread on the aminopterin plate had been grown without thymine, each individual *thy*$^-$ colony probably arises from an independent mutational event. A number of these resistant colonies are picked, purified by streaking on a suitable enriched medium, and then tested for thymine dependence.

As an example, data from the isolation of *thy*$^-$ mutants from χ434 (a derivative of K-12-112) and from strain B/2 are summarized in Table 1. The data from a single plate are presented in each case. Only the largest colonies were picked; large numbers of smaller colonies were seen, as would be expected if the mutational events occur randomly during the growth of the background lawn. The B/2 strain required 48 hr of incubation before the colonies appeared.

This technique has been used without modification to isolate thymine-requiring derivatives of a number of other *E. coli* K-12 and *E. coli* B strains. The stability of the isolates is rather variable as in the original technique, but the large number of independent mutants which this procedure provides makes the selection of stable mutants easier.

TABLE 1. *Summary of thy*$^-$ *selection from Escherichia coli strains*

Strain	Viable cells applied to aminopterin plate	No. of colonies tested	Thymine auxotrophs stable through one purification
χ434	3.8×10^8	100	85
B/2	1.3×10^8	20	9

The aminopterin plates remain usable for at least several months in cold storage.

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