Relation Between Different Thymineless Mutants Derived from *Escherichia coli*

R. R. ROEPKE

Department of Pharmacological Research, Lederle Laboratories, American Cyanamid Company,
Pearl River, New York

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A thymineless mutant strain (70-462) was isolated from a suspension of *Escherichia coli* 15 (ATCC 9723) after X-ray treatment (R. R. Roepke and F. E. Mercer, *J. Bacteriol.* **54**:731,

amount of thymine required by the original mutant. This has been reported by A. P. Harrison (*J. Gen. Microbiol.* **41**:321, 1965) to occur also with thymineless mutants of both *Aerobacter aerogenes* and *E. coli* 15 isolated by the aminopterin method.

A C Broth (Difco), which was used in isolating and maintaining our mutant strains of *E. coli*,

FIG. 1. Growth response (relative turbidity) of mutant 70-462 to thymine in basal medium (*C. M. MacLeod, J. Exptl. Med.* **72**:217, 1940) supplemented with 0.05% Proteose Peptone No. 3 (Difco). Inocula of about 10^4 viable cells per milliliter. T^+ = parent *E. coli* 15. Turbidity was measured with a photomicrorefractometer as described by R. L. Libby (*Science* **93**: 459, 1941).

1947). This mutant, available from the American Type Culture Collection as ATCC 9723h (previously designated ATCC 11,117), proved to be unstable, in that moderately prolonged incubation in growth-limiting concentrations of thymine usually gave rise to a secondary growth (Fig. 1). With few exceptions, this overgrowth was the result of a second mutation to a strain (70V-462) capable of growing with less than one-tenth the

TABLE 1. Incidence of 70V-462 (VT^-) cells in cultures of thymineless strain 70-462 (T^-) grown in the presence of excess thymine^a

Expt	Inoculum (T^- cells/tube)	After incubation		
		Total cells/tube	VT^- cells/tube	VT^- /total cells
I	10^5	1.6×10^9	6.3×10^3	$1/2.5 \times 10^6$
II	10^5	1.4×10^9	1.0×10^4	$1/1.4 \times 10^5$
III	230	4.7×10^9	1.8×10^3	$1/2.7 \times 10^6$
IV	230	7.2×10^9	1.8×10^3	$1/4.1 \times 10^6$

^a Single-colony cultures of 70-462 were inoculated into tubes containing 40 ml of double-strength A C Broth and incubated at 37 C until turbidity reached 10 to 15% of maximum. Cultures were cooled in an ice bath and centrifuged; packed cells were washed twice with cold saline and resuspended in saline. Total viable counts were determined by plating in A C Broth and 2% agar. Viable counts of VT^- cells were determined in Trypticase Soy Agar.

contains sufficient thymine to support growth of mutant 70-462 comparable to that of the parent *E. coli* 15. Other broth media, particularly Nutrient Broth (Difco) and Trypticase Soy Broth (BBL), support only a very light growth of strain 70-462.

Trypticase Soy Broth supplemented with 15 μ M thymine and 1.5% agar was used to determine the number of 70V-462 cells in the presence of large numbers of cells of 70-462. When increasing numbers of washed cells harvested from cultures of strain 70-462 were plated in 10 ml of

this medium and incubated for 30 to 48 hr, the number of visible colonies was relatively constant (averaging 22 per plate) with inocula ranging from 10^5 to 10^6 or more viable cells per plate. With very few exceptions, these colonies were of 70V-462 cells, apparently the result of mutations occurring during limited growth of strain 70-462 in this medium. With larger inocula, the number of visible colonies tended to increase linearly with the number of cells plated, reflecting the presence of 70V-462 cells in the inocula. In this range, the number of colonies less 22 (the average number of mutations occurring in each plate) was taken as the number of 70V-462 cells present in the inoculum. This method was used to determine the incidence of 70V-462 cells in cultures of strain 70-462 grown with excess thymine (Table 1). The higher incidence of 70V cells obtained in experiment II may have resulted from the presence of a 70V cell in the original inoculum in A C Broth. Results of the remaining three experiments indicate a mutation

rate of about 3×10^{-8} as calculated by the method of S. E. Luria and M. Delbrück (*Genetics* 28:491, 1943). The incidence of reverted cells, capable of growth without thymine, was examined in other cultures of 70-462 grown in A C Broth and appeared to be less than 2% of the incidence of 70V cells.

The thymineless mutant designated 15T⁻ by H. D. Barner and S. S. Cohen (*J. Bacteriol.* 68:80, 1954) is a double mutant derived from strain 70-462. The original thymineless strain was supplied to Barner and Cohen by Joseph Gots, who had obtained it from the American Type Culture Collection as ATCC 11,117 (J. Gots, *personal communication*). Thus, strain 15T⁻, strain I of S. Zamenhof and G. Griboff (*Nature* 174:307, 1954), and strain 70V3-462 used by T. R. Breitman and R. M. Bradford (*Biochem. Biophys. Res. Commun.* 17:786, 1964) are similar although not necessarily identical double mutants derived from the same mutant strain, 70-462.