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Elective Selection of Proline-requiring Mutants

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We recently discovered a method whereby proline-requiring (*pro*) mutants of some strains of *Escherichia coli* K-12 can be selected at high frequency (Table 1). The method seems to involve the general principle of differential growth rates. Only *pro E. coli* K-12 mutant strains grew in Penassay Broth (Difco) containing 1 to 3 μg/ml of 4-nitropyridine-*N*-oxide (4NPO), 10 μg/ml of 4-nitrosopyridine-*N*-oxide, or 50 μg/ml of 4-hydroxyl-aminopyridine-N-oxide, whereas strains not requiring proline (pro^+) were inhibited.

The method can be illustrated by the results obtained with a pro^+ *E. coli* K-12 strain, W3630. A single colony of this strain was inoculated into 10 ml of Penassay Broth and incubated at 37 C overnight, during which time growth usually reached a level of about 5×10^8 cells per milliliter. The cell suspension was diluted 10^{-3}, and 0.2 ml was added to 100 ml of Penassay Broth along with 0.2 to 0.5 ml of a solution of 1 mg of 4NPO/ml. This mixture was incubated on a reciprocal shaker at 37 C for 20 to 26 hr, and 0.1 ml of the culture was diluted and plated on eosin-methylene blue-glucose-agar medium (J. Lederberg, Methods Med. Res. 3:55, 1950). Colonies appearing on this medium then were replica-plated onto Davis's minimal (DM) agar with or without 40 μg/ml of L-proline. The percentage of *pro* mutants present in 4NPO-treated cultures is shown in Table 1. Pretreatment of bacteria with a mutagen, such as ultraviolet light or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, increased the frequency of appearance of *pro* mutants.

A preliminary analysis of the method has been made with the following results: *pro* mutants are the only ones selected by this method at a detectable frequency; *pro* mutants of independent origin grow on DM agar containing the metabolic precursors of proline, glutamic-γ-semialdehyde or Δ1-pyrroline-5-carboxylic acid, but they do not grow on DM agar containing glutamic acid (presumably the mutants have a metabolic block between glutamic acid and glutamic-γ-semialdehyde); the mutants produced are stable; conjugation experiments indicate that the *pro* marker is located on the chromosome between the arabinose (*ara*) and lactose (*lac*) loci, close to *lac*. Re-

TABLE 1. *Selection of proline-requiring mutants from Escherichia coli K-12 W3630*

Concn of 4NPO	No. of *pro* mutants*				
μg/ml					
0	0	0	0	0	0
1.0	18.5	8.9	38.5	0.9	17.9
2.0	59.3	53.3	100		
3.0	93.9	1.7	0	0	

* Each culture contained about 10^4 to 10^8 cells per milliliter; the number of colonies tested in each experiment was about 200. Results are expressed as: (number of *pro* mutants)/(number of colonies tested) × 100.

construction experiments with a mixture of *pro* lac^+ and pro^+ lac^- cells showed that the appearance of *pro* mutants was not due to mutation induced by 4NPO, but rather to selective growth in the media.

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