Elective Selection of Proline-requiring Mutants

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Received for publication 3 January 1966

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-hydroxy-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 × 10⁶ 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-nitroso-guanidine, 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-pyroline-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. 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.

We thank R. Sato for fruitful discussions, and Y. Izumi for a gift of glutamic-γ-semialdehyde dimethyl acetal. We also thank S. Kanahara, A. Oiwa, and N. Hiyoishi of the Faculty of Pharmaceutical Sciences, Kanazawa University, for preparation of 4NPO and its derivatives.

This investigation was supported by Public Health Service research grant GM 08293 from the Division of General Medical Sciences.