

## A Method For Assaying and Studying Induced Mutations In Bacteria

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Several methods have been devised for measuring induced mutation rates. They include such principles as the fluctuation test (2), the average number of mutants per culture in a series of parallel cultures (3), clonal systems and population dynamics (1), and serial dilution methods (4).

It has been recognized that after subjecting bacteria to mutagenic action it is necessary to allow a number of divisions on an enriched medium to permit the complete expression of the induced mutants. During studies that were undertaken to determine the effects of various mutagens on different genetic loci, we have attempted to develop a method of sub-culturing which would eliminate many of the time-consuming procedures, such as centrifugation and washing, that are employed when studying auxotrophic mutants.

The proposed method of sub-culturing was incorporated in a procedure, similar in some respects, to that employed by Luria and Delbruck (2), and Pratt, *et al.*, (4), for measuring induced mutations.

At various time intervals, aliquots were removed from the mutagen-treated cell suspension and diluted to a predetermined dilution in order for approximately one-half of the subcultures to contain mutants and the other half not. When assaying for more than one mutation it is sometimes necessary to use two different dilutions, since the frequencies of mutations may not be identical. Total counts were made simultaneously.

The subcultures were made by placing 0.1 ml. samples of the dilution on squares of sterile filter papers on the surface of large plates of brain-heart infusion agar. Two non-irradiated controls were run. One was inoculated with a larger number, and the other with a smaller number of cells than the subcultures. From these controls it is possible to obtain a more accurate spontaneous rate, and also observe if the inoculum size exerts any effect on selection.

The large plates were incubated 18 hours at 37 C. to allow complete phenotypic expression. At this time the paper strips were transferred with sterile forceps to tubes containing 10 ml. of sterile water. The tubes were thoroughly shaken and 0.1 ml. aliquots spread over an area of approximately one square inch on the surface of large plates containing the desired selective medium. After 24 hours incubation at 37 C. the spots were observed visually to determine the number of positive subcultures, those containing more mutants than the controls, or the negative subcultures which possessed approximately the same number of mutants as the controls.

By assuming a Poisson distribution, the number of potential mutants which give rise to mutant clones can be estimated from the dilution factor and the fraction of subcultures containing no induced mutants when compared with the spontaneous level of the controls.

Experiments were conducted to determine the time required for complete phenotypic expression of induced mutants. The results indicated that for several mutations the phenotypic expression was complete at 5 hours, since the same number of mutants was detected at 5 hours as at 24 hours.

This method also facilitates the assaying of large populations simply by increasing the number of filter paper subcultures which entails very little extra work. A number of different selective media were employed. The carry-over of nutrients from the tubes of sterile water was

so small that no interference was encountered when aliquots were assayed for mutants. A satisfactory recovery of mutants was demonstrated when the method was compared with more laborious procedures.

In calculating the mutation rate, by assuming a Poisson distribution, visual observations may be made since the important factor is whether the subculture does, or does not, contain mutants in excess of the spontaneous level. The actual number of mutants in each subculture can be neglected. Since only a few generations in the subculture are necessary for mutants to be detected, this method eliminates errors that may arise because of differences in the growth rates of the mutants, which may affect their becoming established in the total population.

The proposed method for measuring induced mutation rates eliminates many of the time consuming processes employed in other methods, allows the detection of a large number of different mutations by using the appropriate selective media, permits the assaying of large populations which would facilitate the detection of rare mutations, and yet is effective enough to allow the complete phenotypic expression and recovery of induced mutants.

#### LITERATURE CITED

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