

# Use of Dihydrostreptomycin for the Isolation of Auxotrophic Mutants of *Pseudomonas* *aeruginosa*

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Received for publication 17 November 1965

The penicillin screening method (B. D. Davis, *J. Am. Chem. Soc.* **70**:4267, 1948; J. Lederberg and N. D. Zinder, *J. Am. Chem. Soc.* **70**:4267, 1948) has already led to rapid progress in genetic and biochemical research on penicillin-sensitive bacteria. Research on penicillin-insensitive species is also desirable since, for example, there are significant species differences, such as those observed for transfer ribonucleic acids (T. Ishida and K. Miura, *J. Mol. Biol.* **11**:341, 1965). The possibility of isolating auxotrophic mutants from one penicillin-insensitive strain of *Pseudomonas aeruginosa*, strain 62SB2120, which utilizes hydrocarbons as a carbon source (S. Horiguchi et al., *unpublished data*), was inferred from the results of experiments in which dihydrostreptomycin was found to be more bactericidal for growing cells than for resting cells of this strain under suitable conditions. Lederberg and Zinder reported that streptomycin does not alter the mutant ratio of *Escherichia coli* populations.

This note presents a technique for isolating mutants by use of dihydrostreptomycin. Cells of *P. aeruginosa* 62SB2120 were irradiated with ultraviolet light from a Matsuda 15-w germicidal lamp at a distance of 40 cm for 10 sec, to reduce

the viable count from  $10^7$  to  $10^4$  viable cells per milliliter. The irradiated cells were stored in the dark for 2 hr, and then were grown in nutrient broth (Difco) at 30 C for 6 hr. After washing, the cells were incubated in Gray and Tatum's minimum medium (M medium; F. J. Ryan and L. K. Schneider, *Genetics* **34**:72, 1949) at 30 C for 2 hr, then exposed to dihydrostreptomycin (400  $\mu\text{g}/\text{ml}$ ) in M medium at 30 C for 7 hr, and finally plated on nutrient agar (Difco).

In one experiment, 26 auxotrophic mutants were obtained from 385 colonies isolated on nutrient agar, whereas only 1 mutant was obtained from 1,870 colonies isolated on nutrient agar without dihydrostreptomycin treatment. Specific requirements for the nutritional mutants isolated by using dihydrostreptomycin were determined by the auxanographic technique.

We have no evidence that dihydrostreptomycin is mutagenic for this *Pseudomonas* strain, since no mutants were obtained from 10,000 colonies isolated on nutrient agar after treatment with dihydrostreptomycin without ultraviolet irradiation. Not all the mutants isolated by this technique were resistant to dihydrostreptomycin.