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Enriched Selection of Dominant Mutations: Histidine Operator Mutations

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In the course of selection of bacteria with derepressed levels of histidine biosynthetic enzymes, it was found that when mutagen-treated cells were spread on a selective medium without allowing intervening growth to occur, the frequency of operator mutants obtained was dramatically increased. This may be useful as a general enrichment for operator or other dominant mutations.

Of the six classes of mutations resulting in the constitutive production of high levels of the histidine biosynthetic enzymes, mutation of the operator class is the rarest. Only three of the nearly 200 constitutive mutants originally isolated by Roth, Antøn, and Hartman (2) are operator mutants. In those experiments, the mutagenized bacteria were grown for several generations before being spread on the selective medium. We have now found that the proportion of operator mutations is increased dramatically if bacteria are subjected to selection directly after mutagenesis.

The experiments to determine the effect of gene expression were done in *Salmonella typhimurium* grown aerobically at 37 C. Minimal medium was the E medium of Vogel and Bonner (5) supplemented with 1% glucose. Complete medium was 0.8% nutrient broth with 0.4% NaCl. Tubes containing 2 ml of minimal medium were inoculated with either single clones or from 10 to 100 cells of wild-type *Salmonella typhimurium* LT-2. When these cultures were fully grown (about 4×10^9 cells/ml), they were shaken with 0.05 ml of diethylsulfate for 10 min at 37 C. A 0.05-ml amount of each mutagenized culture was then immediately spread on solid medium containing 20 mM 3-amino-1,2,4-triazole for the selection of mutants constitutive for histidine biosynthesis (2). After the culture was spread, a few crystals of 1,2,4-triazole-3-alanine were added to the surface of the plate. Another 0.05-ml portion was allowed to grow overnight to allow time for gene expression in 5 ml of either the minimal or the complete medium. Those cultures that were grown on the complete medium were washed and resuspended in an equal volume of minimal medium before being plated on the solid selective medium. As before, 0.05 ml of this washed cell suspension was spread on selective medium. After 2 to 5 days, the analogue-resistant colonies were picked and purified by further streaking on solid minimal medium containing 2% glucose. Colonies of constitutive bacteria were recognized by their characteristic wrinkled morphology on the plates supplemented with 2% glucose (3).

Histidine operator (*hisO*) mutants were identified by transduction with the non-lysogenizing mutant, *int4*, of phage P22 (4) by using the following procedure. Constitutive colonies were transferred with sterile applicator sticks to tubes containing 2 ml of complete medium. After these cultures reached a cell density of 10^8 to 2×10^8 cells/ml, 5×10^6 phage were added, and growth was continued for about 12 hr. The lysed cultures were then shaken with a few drops of chloroform to kill the bacteria. About 0.01 ml of the resulting phage preparation was spotted onto a plate of solid minimal medium which had been previously spread with the recipient strain, *his-*

TABLE 1. *Operator-type mutants among constitutive bacteria obtained*

Expt	Gene expression allowed	No. of constitutive bacteria analyzed	No. of *hisO* mutants	Per cent of *hisO* mutants
1	No	166	77	46
	Yes	72	1	1
2	No	82	14	17
	Yes	70	2	3
3	No	97	30	31
	Yes	292	33	11

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OG203. Since the *hisO* region of the mutant *hisOG203* is deleted, all His$^+$ transductants carry the operator region of the donor strain. Thus, all of the transductants are either wrinkled or smooth, depending on whether the transducing phage had been grown on a *hisO* mutant. Twenty mutants could be tested easily on a single petri dish.

The results of three series of experiments performed several months apart are summarized in Table 1. In each experiment, subjecting cultures to selection immediately after mutagenesis resulted in a dramatic enrichment of operator-type mutants among the constitutive bacteria obtained. It is important that the bacteria are grown in minimal medium before mutagenesis. In several experiments where they were grown in complete medium before mutagenesis and then plated directly on minimal medium, there was no enrichment for operator mutants. This could be due to the added complication of the shift from rich to poor medium.

The enrichment for operator mutants presumably reflects the fact that the operator gene does not code for a cytoplasmic product (1). Therefore, mutations in the operator gene may be expressed immediately, despite the presence of wild-type cytoplasm or other wild-type nuclei in the cell. The five other histidine regulatory genes, on the other hand, are recessive (1) and code for diffusible products. The constitutivity of these other mutants is not expressed until dilution of the wild-type cytoplasm and segregation of mutant nuclei has occurred. If mutagenized cells are allowed to grow for several generations before being spread on the selective medium, these five non-operator mutant types can be expressed and represent the vast majority of the constitutives selected. However, the fraction of bacteria that are operator types is not increased by the gene expression; as a result, the fraction of operator mutants among the constitutive bacteria is greatly reduced.

Presumably exposing bacteria to suitable selective conditions immediately after mutagenesis will allow the enriched selection of operator-type mutants in other systems, and, more generally, of bacteria carrying dominant mutations.

This may be useful as a general enrichment for mutations which are immediately expressed.

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