Modification of EMS-induced reversion frequencies in Neurospora with enriched plating media

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Abstract
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Modification of EMS-induced reversion frequencies in Neurospora with enriched plating media.

It is well known that ultraviolet mutation frequencies may be modified by various post-treatment nutritional conditions. Maling (personal communication) has found that the nitrous acid-induced reversion frequency of certain ad-3 Neurospora mutants may be enhanced by plating on medium supplemented with low levels of adenine. It was considered of importance, therefore, to ascertain the effects of several enriched plating media on the recovery of reverse mutations following exposure to ethyl methanesulfonate (EMS).

Conidia from an ad-3 mutant of Neurospora were uniformly treated with EMS and plated on minimal medium supplemented with various levels of adenine, and minimal medium containing casamino acids supplemented with various levels of adenine.

From the results obtained, we may draw the following conclusions: (1) Following EMS exposure, plating conidia on minimal medium plus casamino acids enhances the observed mutation frequency two-fold over that seen on minimal medium. (2) The addition of low levels of adenine to minimal medium also increases the frequency of recovered mutations by a factor of 2. (3) The addition of low levels of adenine to minimal medium supplemented with casamino acids has very little or no effect.

These results establish that the recovery of EMS-induced revertants may be modified quantitatively by plating the conidia immediately following treatment on enriched media. While superficially these findings are similar to those obtained in bacteria, at this time we cannot conclude that similar mechanisms are involved.

From Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

Carsiotis, M. and A. Lacy. Tryptophan synthetase levels in hist-1 mutants. 401 (1952). The equivalent mutant of S. typhimurium has been found to produce normal levels of tryptophan synthetase.

Gross, S. R. A selection method for mutants requiring sulfur-containing compounds for growth. Experiments designed to detect reversions and suppressors of leucine-4 mutants of Neurospora have consistently yielded microcolony forming "pseudo-prototrophs". The "pseudo-prototrophs" have invariably proven to be double auxotrophs requiring for growth some specific sulfur-containing compound as well as leucine. Thus far 14 leu-4 mutants of independent origin and distinctive complementation patterns have been examined, and, in each case, sulfur-requiring mutants have been obtained. Five of these mutants have been examined for specific growth factor requirements. Four have been found to be different; sulfite dependent, cysteic acid dependent, cysteine dependent, and one that would respond to cysteine, homocysteine or thiosulfate (slightly). All of the mutants save the cysteic acid requirer respond to methionine. The spontaneous frequency of "pseudo-prototrophs" has been as high as 1 x 10^-5 but depends greatly on the leu-4 mutant employed.

All of the sulfate mutants obtained thus far have appeared as suppressors of leu-4 when selection for revertants was carried out on minimal medium. The auxotrophs obtained are therefore quite leaky for their sulfur requirement. Selection for completely blocked sulfur mutants on sulfur-containing medium devoid of leucine seems doomed to failure for the following reason: The suppression of leu-4 depends to a large extent on the diminution of the synthesis of some sulfur-containing compound. The suppression is completely