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A selection method for mutants requiring sulfur-containing compounds for growth

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Abstract

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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 1×10^{-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

eliminated when sulfur compounds required for growth are added to the minimal medium. The method therefore seems useful for obtaining a large class of sulfur-requiring auxotrophs with partial losses of enzymatic function.