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Salmonella typhimurium hisG46 (R-Utrecht): Possible Use in Screening Mutagens and Carcinogens

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Salmonella typhimurium LT2 *hisG46* becomes a more sensitive strain for assaying mutagens and carcinogens when it carries the resistance transfer factor R-Utrecht.

Ames (1, 2) described four strains of *Salmonella typhimurium* for detecting and classifying mutagens and carcinogens. Each auxotrophic strain (histidine requiring) has been selected for its sensitivity and specificity and can be reverted back to the wild type by particular mutagens. In addition, the strains have a deleted excision repair system and, consequently, are very much more sensitive to various mutagens. This paper describes another way in which one of the parental (i.e., repair proficient) histidine-requiring strains (*hisG46*) can be made more sensitive to various mutagens and reports the detection of a possible mutagenic compound which otherwise escapes detection in Ames' system.

The strains used, *S. typhimurium* LT2 *hisG46* and TA1530, were kindly provided by B. N. Ames. TA1530 is a strain containing the *hisG46* mutation and also a deletion through a gene involved in the excision repair system for deoxyribonucleic acid (*uvrB* gene; see reference 1). Strain LT2 *hisG46* has a base substitution which alters one codon in the messenger ribonucleic acid from the gene coding for the first enzyme of histidine biosynthesis and can be reverted to wild type by agents such as ultraviolet light which cause base pair substitutions (1). I have found that, in common with several other strains of *S. typhimurium*, the susceptibility of strain LT2 *hisG46* to the mutagenic effects of ultraviolet light is considerably enhanced when it carries the drug-resistance transfer factor R-Utrecht (see reference 3). It therefore seemed worthwhile to test a known chemical mutagen for its effect on reversions in strain LT2 *hisG46* with and without the R-Utrecht factor.

The method used has been described by Ames (1). Overnight cultures of the strains to be tested (0.1 ml) were added to 2 ml of 0.6% agar containing 0.5% NaCl, and the resulting mixtures were poured onto the surface of minimal agar plates containing a trace of histidine (0.02 μg/ml) and an excess of biotin (0.025 μg/ml). Drops (10 μliters) of the mutagen methyl methane sulfonate (MMS) diluted in phosphate-buffered saline (pH 7.0) were then placed on the surface of the plates, and the plates were incubated at 37 C for 3 days. In both LT2 *hisG46* and LT2 *hisG46* (R-Utrecht), the addition of between 7.5 and 45 μmol of MMS caused the appearance of large numbers of revertant colonies in a ring around the spot of mutagen. On addition of 6 μmol of MMS, two to three times as many revertants of the R^+ strain were found, whereas on addition of 3 or 4.5 μmol, only the R^+ strain yielded revertant colonies in a ring around the spot of mutagen. The R-Utrecht factor thus appeared to increase the susceptibility of strain LT2 *hisG46* to the mutagenic action of MMS and might therefore be expected to increase its susceptibility to the mutagenic effects of compounds which act in a similar way.

To determine whether this property of R-Utrecht might be of some practical value, I tested a range of compounds for possible mutagenicity in strains LT2 *hisG46* and LT2 *hisG46* (R-Utrecht). Of 27 compounds taken at random from the laboratory shelves, one (trimethyl phosphate, a petroleum additive) was found to cause the appearance of revertant colonies close to the spot of application in strain LT2 *hisG46* (R-Utrecht) but not in strain LT2 *hisG46* (Fig. 1). Moreover, this effect of trimethyl phosphate could not be demonstrated with strain TA1530 despite the increased susceptibility of this strain to certain other mutagenic compounds. It therefore appears that a

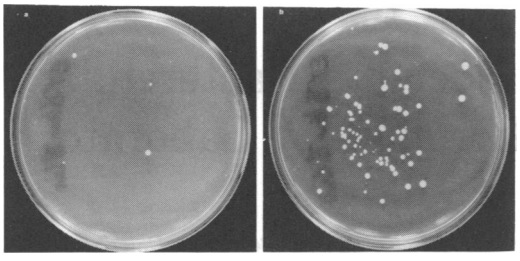

FIG. 1. *Plate test for induction of revertants in histidine-requiring strains of S. typhimurium by trimethyl phosphate. Drops (70 μmol) were added to the centers of plates seeded with (a) strain LT2 hisG46, and (b) strain LT2 hisG46 (R-Utrecht).*

wider range of mutagens capable of causing base-pair substitutions will be detected if strain LT2 *hisG46* (R-Utrecht) is used in addition to strain TA1530 in Ames' system.

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