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Selection of 5-Methyltryptophan-Resistant Adenine Auxotrophic Cell Line of *Datura innoxia* MILL

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Cell lines resistant to amino acid and nucleic acid analogs from cultured cells of higher plants has a potential use in studies of plant metabolism and genetic manipulation. A number of resistant cell lines of various plant species to date have been estabished.[1-6] However, among these resistant cell lines there are only a few instances[7,8] of a double mutant which has an auxotrophic nature and is resistant to a toxic chemical, even though such a mutant has a very useful potential in studies of somatic hybridization.

Lo Schiavo et al.[7] isolated a carrot cell mutant resistant to both 8-azaguanine and α-amanitin. This double mutant can be used as a universal hybridizer since the somatic hybrids between the mutant and any wild-type cell could be selected by eliminating the mutant strain with HAT medium[9] and the wild-type cell with α-amanitin. Recently, we isolated a strain resistant to 5-methyltryptophan (5-MT) from adenine auxotrophic cells (Ad^- strain) of *Datura innoxia* MILL. This strain can be used, as a universal hybridizer, for studies of somatic hybridization. We report here some characteristics of the 5-MT-resistant cells.

The adenine auxotrophic strain used in this experiment was originally isolated by King et al.[10] and supplied to our laboratory through his generosity in 1982. The strain was maintained on the B5A solid medium[10] supplemented with 80 $\mu g \cdot ml^{-1}$ of adenine.

Selection of the strain resistant to 5-MT was carried out in a stepwise manner. Cells that grew slowly in the presence of 10 $\mu g \cdot ml^{-1}$ of DL-5-MT and 80 $\mu g \cdot ml^{-1}$ of adenine were subcultured on the same medium for 2 months and transferred to the medium containing 20 $\mu g \cdot ml^{-1}$ of 5-MT. After selection by culturing on the same mdium for 3 months (4 transfers), adapted cells were transferred to the medium containing 50 $\mu g \cdot ml^{-1}$ of 5-MT. Selection was continued on the medium containing 50 $\mu g \cdot ml^{-1}$ of 5-MT for a half year (8 transfers). Adapted cells were maintained for one year (20 transfers, about 53 cell mass doublings) on the B5A solid medium containing 50 $\mu g \cdot ml^{-1}$ of 5-MT and 80 $\mu g \cdot ml^{-1}$ of adenine. The auxotrophic phenotype of the established strain was confirmed to be stable through this selection process.

The resistant cells (Ad^- 5 MT^r) were found to be resistant up to 100 $\mu g \cdot ml^{-1}$ of 5-MT, whereas the growth of the non-adapted cells was completely inhibited at 10 $\mu g \cdot ml^{-1}$ of 5-MT (**Fig. 1**). The growth curve of the Ad^- 5 MT^r indicates about 9 fold increase in fresh weight during 3 weeks of culture (data not shown).

Widholm[11,12] and Sung[13] demonstrated that 5-MT apparently inhibited growth of wild-type tobacco and carrot cells by inhibition of anthranilate synthetase so that the cells died for tryptophan starvation. Growth inhibition by 5-MT of both Ad^- 5 MT^r and Ad^- strains was reversed by supplementation of tryptophan to the medium, although tryptophan tended to inhibit growth of the Ad^- strain (**Fig. 2**). This suggests a possibility that 5-MT also inhibited anthranilate synthetase in *Datura* cells and that this leads to a tryptophan starvation.

The content of endogenous free tryptophan was 6 fold greater in Ad^- 5 MT^r cells than in Ad^- cells, although the extent of increase was smaller than that in tobacco 5-MT-resistant cells.[14] How-

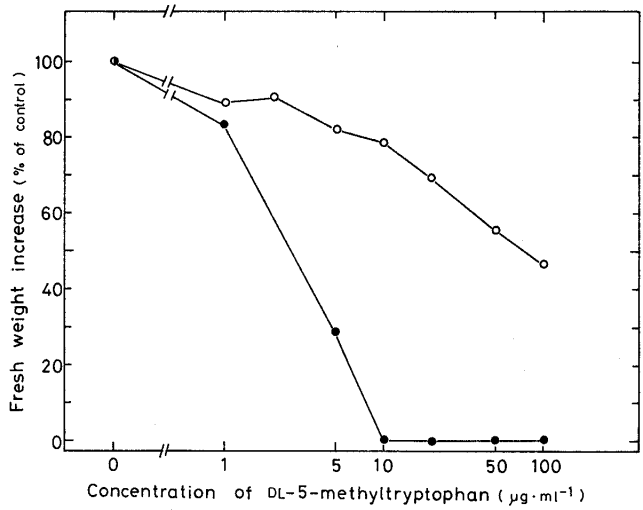

Fig. 1. Effect of DL-5-MT on the growth of resistant Ad⁻ 5 MT^r cells (○) and non-resistant Ad⁻ cells (●). The callus cells of 0.2 g in fresh weight were divided into 4 small pieces (about 50 mg) and inoculated on 25 ml of solidified B5 A medium (0.8% agar) containing 80 $\mu g \cdot ml^{-1}$ of adenine and various concentrations of DL-5 MT in a 50 ml conical flask. The cultures were incubated at 26±1℃ in the dark. After 14 days incubation the callus cells were collected and weighed their fresh weights.

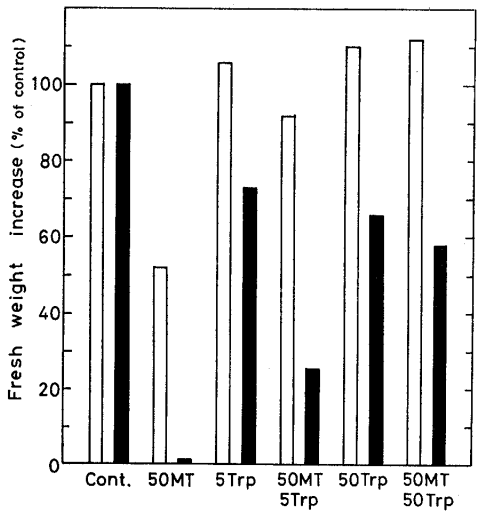

Fig. 2. The reversal of the 5-MT inhibition of growth by tryptophan. Measurements were recorded after 14 days of growth on B5 A solidified medium containing 80 $\mu g \cdot ml^{-1}$ of adenine (control medium) and 5-MT or/and L-tryptophan of the indicated concentrations. 50 MT, 5 Trp and 50 Trp indicate 50 $\mu g \cdot ml^{-1}$ of 5-MT, 5 $\mu g \cdot ml^{-1}$ of tryptophan and 50 $\mu g \cdot ml^{-1}$ of tryptophan, respectively. The culture conditions were the same as in **Fig. 1.** □: Ad⁻ 5 MT^r cells, ■: Ad⁻ cells.

ever, unlike the case of carrot 5-MT-resistant cells,[13,14] the growth of auxin-indepentent callus was not observed in these Ad$^-$ 5 MT^r cells.

The stability of resistant phenotypes in culture without selection is variable. Resistant cell lines to the herbicides, picloram[15] and glyphosate[16] continue to exhibit their resistance for long periods without selection. In contrast, the resistance to cycloheximide is very unstable in cell lines of tobacco[17] and carrot.[18] Various degrees of the stability have been reported in cell lines resistant to amino acid analogs.[12,19-24] The resistant phenotype of the *Datura* cells, Ad$^-$ 5 MT^r, has been maintained at least for 4 months (8 transfers, about 28 cell mass doublings) in culture on the medium lacking 5-MT.

Detailed characterization of Ad$^-$ 5 MT^r cells and single cell cloning is underway in our laboratory.

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Datura innoxia の 5-メチルトリプトファン耐性でアデニン要求性細胞の選抜

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Datura innoxia アデニン要求性培養細胞から，5-メチルトリプトファン (5-MT) 耐性株を選抜した．耐性株は，5-MT (50 μg/ml) を含む培地において，3週間で9倍に生長するが，オーキシン欠除の培地では，まったく生長できなかった．耐性は，5-MT 欠除の培地で，少なくとも4カ月は維持された．