Gene-controlled resistance to aromatic hydrocarbons Neurospora crassa and its relationship to the inhibition by L-sarbose

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Abstract
Resistance to aromatic hydrocarbons
supplied by a 15-watt Sylvania soft-white fluorescent tube. One milliliter of the treated conidial suspension was placed in each of twenty petri dishes and mixed with Neurospora minimal agar (Difco) supplemented with sorbose (8 gm./l.) to induce colony formation. The plates were incubated at 27°C for 48 hours before reading. Survivors were counted and compared with the number of survivors on an non-photoreactivated plates.

The five-minute exposure to white light was previously determined to bring about maximum photoreactivation in the strain of Neurospora used. Six treatments were used, each carried out with 1.32 x 10⁶ conidia dispersed in each of 20 plates. The first group was treated with UV but not reactivated. The second group was treated with UV and immediately reactivated. The third was irradiated and incubated for five minutes before reactivation. The other three groups were irradiated and incubated for 30, 60, and 90 minutes respectively before photoreactivation.

The conidial samples assayed for DNA level spectrophotometrically were allowed to incubate for varying periods, as mentioned above, after irradiation and before treatment. The same periods were used for both methods of DNA measurement. The absorbancy due to the DNA color reaction at each delay interval is compared with the mutation rate following photoreactivation at each interval in the following graph. A close correlation between DNA level as measured spectrophotometrically and by the post-photoreactivation mutation rate is demonstrated. — — Department of Biological Sciences, Kent State University, Kent, Ohio 44240 and Eli Lilly and Co. Research Laboratories, Indianapolis, Indiana 46206.

Georgopoulos, S.G., A. Kappas and B. Macris. Gene-controlled resistance to aromatic hydrocarbons in Neurospora crassa and its relationship to the inhibition by L-sorbose. Vomvayianni 1965 Can. Jour. Bot. 43:765). Five such strains were used in random and tetrad analyses and each was shown to have resulted from a single-gene mutation. There may be more than one mutational site for resistance to these hydrocarbons as it has been shown for other fungi (Currs et al. 1956 Am. J. Botany 43:594. Whittingham 1962 Am. J. Botany 49:866, Georgopoulos and Kappas 1966 Con. Jour. Genet. 8:347). At least one of these sites is linked to the mating type locus and to patch (see also NN9:44), On control medium hydrocarbon resistant strains tend to sporulate less abundantly than the respective wild types.

Although patch confers no tolerance to the hydrocarbons all hydrocarbon resistant mutants “escape” the effect of L-sorbose as least as effectively as patch. On media containing sucrose and L-sorbose some of these mutants grow much better than Patch. Whether different levels of inhibition by sorbose are associated with different genes for resistance to aromatic hydrocarbons is now being investigated. — — Department of Biology, Nuclear Research Center "Democritus", Athens, Greece.

Neurospora crassa strains STA4 (wild type) and patch (monoclonal growth an up to 1% L-sorbose) were used and were found highly sensitive to diphenyl, naphthalene, acetophenone and other similar compounds. Resistant strains were obtained from fast growing sectors on media described for other fungi (Currs et al. 1956 Am. J. Botany 43:594). Vomvayianni 1965 Can. Jour. Bot. 43:765). Five such strains were used in random and tetrad analyses and each was shown to have resulted from a single-gene mutation. There may be more than one mutational site for resistance to these hydrocarbons as it has been shown for another fungal (Currs et al. 1956 Am. J. Botany 43:594). Whittingham 1962 Am. J. Botany 49:866, Georgopoulos and Kappas 1966 Can. Jour. Genet. 8:347). At least one of these sites is linked to the mating type locus and to patch (see also NN9:44). On control medium hydrocarbon resistant strains tend to sporulate less abundantly than the respective wild types.

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Day, C.H. Control of aromatic biosynthesis in Neurospora crassa. 3-Deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase (DAHP synthetase) is the first enzyme of aromatic biosynthesis in micro-organisms and in E. coli has been shown to be a regulatory system of at least 3 isoenzymes (Doy and Brown 1965 Biochim. Biophys. Acta 104:377). Control is by feedback inhibition (phenylalanine and tyrosine) and repression (phenylalanine, tyrosine and tryptophan (Brown and Day 1966 Biochim. Biophys. Acta 118:157).

DAHP synthetase has now been examined in dialysed crude extracts of wild type N. crassa.74A, grown an Vogel's minimal medium at 25°C for 48 hrs. Under the conditions stationary phase had not been reached. Extracts were made by grinding with glass and KH₂PO₄-NaOH buffer 0.1M pH 6.4 and dialysing against 0.025M of the same buffer. The supernatant was used after centrifuging the debris. DAHP synthetase was estimated essentially as described by Day and Brown.

The substrates are erythrose 4-phosphate and phosphoenolpyruvate and initial velocity measurements were determined by varying one substrate (10⁻⁵M - 2 x 10⁻³M) in the presence of excess of the other (2 x 10⁻³M). By plotting v against s, sigmoid curves were obtained which, within experimental error, had a positive initial slope. Reciprocal Plots of 1/v against 1/s show the characteristics more clearly. Parts of these data replotted as 1/v against 1/s yield a straight line as required if 1/v against 1/s is a parabola. However, it appears likely that this is fortuitous and that the present data are more consistent with the characteristics of a non-rectangular hyperbola. It is important to make this distinction.

A parabolic 1/v against 1/s curve is consistent with a model: E + S \rightarrow ES \rightarrow K₁ ES \rightarrow K₂ ES + product.