A Neurospora mutant resistant-to 2 deoxy-D-glucose.

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Abstract
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TABLE 1

| Characteristics of N. crassa nuclear and whole cell DNA |
|----------------|----------------|----------------|----------------|
|                 | Whole Cell DNA |                 | Nuclear DNA    |
| Minor Fraction  | Major Fraction  | Minor Fraction  | Major Fraction  |
| Tm °C           | % of total DNA | Tm °C           | % of total DNA |
| Conidia         | 80             | 24              | 93             | 76             |
| Mycelia         | 81             | 25              | 92             | 75             |

These data are summarized from optical melting curves at 0.12 M phosphate buffer, pH 6.8. Tm °C (temperature at which 50% of the DNA dissociates) was calculated for each fraction. G:C content can be calculated by the equation G:C mol% = Tm °C 69.3/0.41.

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A mutant strain of Neurospora crassa has been isolated which is resistant to inhibition by 2-deoxy-D-glucose (2dg) and is characterized by growth rates which are initially faster than wild type strains, when grown on minimal medium supplemented with any one of a number of monosaccharides or disaccharides plus 2dg (saccharide/2dg = 2/1). The strain which has been shown in numerous crosses to segregate as a single gene is designated as dgr. dgr grows more slowly than wild type on standard media. These properties and other experimental results suggest that dgr strains permit the utilization of hexoses in an abnormal manner conferring an increased resistance to 2dg.

The original intention was to screen for mutants with increased cellobiase activity ( HACKEL anti Kahn 1978 Molec. gen. Genet. 164: 295). It was expected that 2dg would inhibit wild type growth on cellobiose, while mutants would grow on such a mixture. To date, no mutants have been found with altered cellobiase levels, but several are clearly resistant to 2dg.

Mutagenesis experiments were carried out using the gluc-2 strain ( Eberhart and Beck 1973 J. Bacteriol. 116: 295), which reduces aryl-β-glucosidase activity and permits a clearer nutritional response to cellobiose by putative mutants. Conidia from seven day old gluc-2 cultures were suspended, filtered through glass wool, washed, and diluted to 106/ml in a final volume of 20 ml in a 100 mm diameter round glass dish. Irradiation for 5 min with a U.V. lamp achieved approximately a 50% kill as determined by subsequent growth on complete medium. One ml of irradiated conidia was added to 4 l of incubation medium in a round 6 l flask.

The incubation medium included Vogel's minimal medium at 1/4 strength, 0.1% cellobiose, and 0.05% 2dg. Agar (0.1%) was included to decrease the fusion by anastomosis. Sterile air bubbles agitated the suspension for 24 h at 25°C, then aliquots were viewed in petri dishes under a stereo-microscope. Larger colonies were removed, washed with sterile water and placed in tubes of complete agar medium. The restricted growth habitat induced by 2dg (Tatum, Barratt, and Cutter 1949 Science 109: 509) greatly facilitated colony isolation.

Confirmation that the dgr mutant is resistant to 2dg was obtained with solid media in petri plates containing 0.1% cellobiose or fructose, 0.05% 2dg, 1.5% agar and 1/4 strength Vogel's minimal at 25°C. dgr conidia germinated and grew in 24 h at 25°C, whereas wild type initially grew very slowly but eventually adapted by 3-4 days. In 2dg medium the saccharides that showed a greater differential growth between dgr and wild type are cellobiose, trehalose, lactose, fructose and galactose. Saccharides that showed a lesser, but definite, effect are maltose, glucose, and xylose.
Figure 1. Growth of wild type (A) and dgr strain (B) at 25°C on Vogel's minimal medium plus: □, 1% glucose; 1% glucose plus 0.5% 2dg; □, 1% fructose; □, 1% fructose plus 0.5% 2dg.

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Amylose in Neurospora.

Neurospora is known to contain glycogen, but no reports indicate the presence of amylose (linear starch) even though Vescodyne stains the mycelia blue-black. Since Vescodyne contains iodine and a blue-black response with iodine is a positive test for starch, we stained Neurospora crassa with iodine solution (0.2% iodine 2% KI). As a blue-black stain iodine salicylate, 1 N NaOH, or boiling water, all of which will dissolve starch from algae and higher plants. None of these solubilized Neurospora starch.

Earlier work (McCracken 1974 Plant Physiol. 54:414) showed the existence of an amylose precipitating factor in fungi. This factor has now been isolated from Neurospora and characterized (unpublished results). In our attempts to extract starch, we found that the blue-black staining material co-precipitated with the amylose precipitating factor. This factor binds only to amylose and not to amyllopectin, glycogen, cellulose, dextran, inulin, or a variety of simple sugars. Moreover, in this case the iodine stain was blue. Thus we conclude that Neurospora does indeed contain starch in the form of amylose. Furthermore, since the blue stain is associated with cell walls, this amylose may be a cell wall component. *Department of Biological Sciences, Illinois state University, Normal, Illinois 61761.


DNA homologies of ribosomal RNA genes of Neurospora species.

Ribosomal RNA genes (rDNAs) of Neurospora crassa contain DNA sequences which code for 17S, 5.8S, and 26S rRNAs, in addition to internal and external spacers (Free, Rice, and Metzenberg 1979 J. Bacte. 137:1219). As has been reported for many eukaryotes, the DNA sequences which code for 17S, 5.8S, and 26S rRNAs in Neurospora species are probably conserved while the internal and external spacer regions are probably variable sequences. Extensive electron microscopic studies (Schibler et al. 1975 J. Molec. Biol. 94:503) of 45S precursor rRNA of several cold and warm blooded animals confirm that spacer regions vary extensively from species to species.

It was desirable to know whether such differences in rDNA sequences exist between Neurospora species. Any such difference should be detectable using standard procedures for DNA homology studies (Dutta 1976 Mycologica 68:388). rDNA sequences were isolated from N. crassa mycelial cells using the procedure described previously (Chattopadhyay et al. 1972 Proc. Natl. Acad. Sci. 69:3256). The purified rDNA was H-labeled (by nick translation) and reassocciated with total DNA isolated from the heterothallic species N. crassa and from three homothallic...