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Abstract

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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 wiid 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 10^6/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 1 of incubation medium in a round 6 1 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 25C, 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.

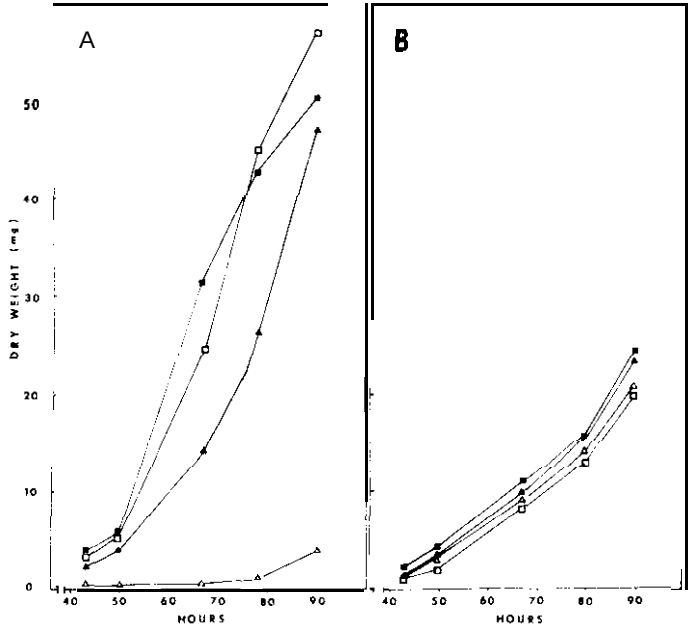

Figure 1. Growth of wild type (A) and dgr strain (B) at 25 C on Vogel's minimal medium plus: ■, 1% glucose; 1% glucose plus 0.5% 2dg; □, 1% fructose;. , 1% fructose; Δ, 1% fructose plus 0.5% 2dg.

Liquid medium was also used to test growth responses. Erlenmeyer 125 ml flasks with 20 ml Vogel's medium and 1% glucose or fructose with or without 0.05% 2dg were inoculated and incubated for 40-100 h at 25 C. Results typical of several experiments (Fig.1 show that: 1) wild type grows better than dgr on fructose or glucose alone; 2) wild type is strongly inhibited in fructose-2dg medium, but not in glucose-2dg medium; 3) &grows poorly on all media; 4) 2dg has a relatively small inhibitory effect on dgr. These results suggest dgr strains have a generally lowered ability to utilize hexoses.

By analogy with yeast, the site(s) of 2dg inhibition in Neurospora may not be easy to define (Kuo and Lampen 1972 J. Bacteriol. 111: 419). The initial steps in glucose and fructose utilization are being examined in Neurospora dgr and wild type strains, following the hypothesis that dgr has a defective step in either uptake or phosphorylation of glucose or fructose. This postulated defect may allow 2dg resistance, because 2dg cannot be effectively converted to an active inhibitory form (possibly 2dg phosphate) the dgr strain. We are seeking other 2-dexoy-glucose-resistant mutants. Genetic analysis is also being completed.

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