

Bates, W. K. Glycerol as carbon source

for induction studies in *Neurospora*.

In studies of enzymes induced by lactose and by galactose in *Neurospora*, it has become apparent that growth conditions must be carefully controlled, and that shaking cultures containing a single carbon source can provide reproducible conditions well-suited for these studies. The ideal carbon source should cause no repressive in-

terference with the induction process. Glycerol is suitable for such studies, but wild type strains are quite variable in their ability to grow on glycerol under the required conditions. The isolate 105-L5-A (formerly designated L5D, Bates and Woodward 1967 *Neurospora Newsl.* 12: 11) shows greatly improved growth on glycerol when compared with STA4. Crosses of this isolate to wild type 74-OR8-1a (Bates 1967 *Genetics* 56: 543) yielded a variety of isolates with improved glycerol growth characteristics, although initial selection was for lactose growth. Two of these were crossed (211-L5-a x 341-8A) and an isolate designated 411-L5-A was obtained. This isolate has been used for all subsequent glycerol growth studies.

Growth conditions are: rotary shaking, 3/4 inch radius, 150 cycles per minute, $30 \pm 0.5^\circ\text{C}$, 0.18 M glycerol, Vogel's medium, 200 ml in 500 ml Erlenmeyer flasks, mounted at a 30 degree angle. The Vogel's medium is autoclaved at 2 x concentration, and the carbon source is autoclaved separately in 100 ml water. The inoculum is 106 conidia per ml medium. Under these conditions growth is linear for ca. 90 hours (yielding ca. 1.5 g dry weight), and comparisons are made by harvesting at 48 hours (yielding ca. 0.7 g dry weight). The rate of growth with glucose under these conditions is ca. two times the rate obtained with glycerol.

In comparison with 411-L5-A, taken as 100%, growth of some wild type strains on glycerol can be grouped in the following way: STA4 and RL-A, 33-36%; ST73 a, RL-a and Em-A (FGSC#691), 58-68%; Em-o (FGSC#692), 103% (all based upon total mycelial dry weight at 48 hrs). RL-A and RL-a are Rockefeller-Lindgren isolates obtained from J. F. Wilson. It is apparent that a mating type shows better glycerol growth than does A for all three strains, except for 411-L5-A. When grown on sucrose under otherwise identical conditions, little difference ($\pm 10\%$) in total growth is observed among these different strains. Another distinguishing characteristic is the orange pigmentation which occurs under these glycerol growth conditions in an inverse relationship to ability to grow on glycerol. The Em-o and 411-L5-A cultures show no evidence of this pigmentation.

Among the isolates obtained along with 411-L5-A, there was a marked correlation between ability to grow on glycerol and reduced production of conidia. For example, 411-L5-A produces only 30-50% of the conidia produced by STA4 when grown and harvested under the same conditions. That this characteristic is not necessarily associated with glycerol growth is shown by Em-o, which conidiates more abundantly than STA4, but which grows well on glycerol. Crosses designed to combine the glycerol growth characteristics with amino acid and inositol requirements are now in progress. The 411-L5-A isolate produces abundant protoperithecia on Westergaard's synthetic cross medium, and up to 90% spore viability, but the mature perithecia apparently have low internal pressure, and discharge ascospores weakly.

It appears that the 411-L5-A amino acid auxotrophs have very similar glycerol growth characteristics, but these studies have not been completed. Such characteristics would allow very precisely controlled studies of incorporation of labeled amino acids during induction studies. If these isolates appear to be potentially useful to other workers, the set of cultures will be deposited in the Fungal Genetics Stock Center. (Supported by NSF Grant GB 5189). - - - Department of Biology, The Univ. of North Carolina at Greensboro, Greensboro, North Carolina 27412.