K K Jho. Growth characteristics of phen-1 mutants.

1. Carbon source ond the response of phen-1 auxotrophs to amino ocidr. Initially phen-1 (H 6196) was reported to use any one of the aromatic amino acids or leucine for growth, in the presence of sucrose (Barratt ond Ogata 1954 Am. J. Botany 41: 763). However, many iroloter were found later that grew very poorly on phenylolanine. Newmeyer (1963 Neurospora Newsl. 4: 10) reported that such iroloter regain the ability to grow well on phenylolanine if glycerol is used as carbon source instead of sucrose. She later found that phen-1 would also grow on serine when glycerol was the carbon source. Since phcn-1 (H6196) does not use serine in the presence of sucrose (Barratt ond Ogata 1954) this observation was checked with another phen-1 strain (H3791) ond it has been found that the strain grew on a wider range of amino acids in media with glycerol (2%) or ribose (2%) as sole carbon source. The strain did not grow on minimal medium, irrespective of the carbon source.

(a) Amino acids which promote growth on glycerol or ribose medium: serine, threonine, isoleucine, valine, leucine, glycine, methionine, phenylolonine, tyrosine, tryptophan.

(b) Inactive (no visible growth) amino acids: olonine, arginine, lysine, glutamic acid, glutamine, aspartic acid, asparagine, histidine, proline, cystine.

Each L-amino acid was tested individually at a concentration of 5 mg/20 ml Vogel medium in a 125 ml Erlenmeyer flask with the exception of L-tyrosine and L-tryptophan which were tested at a concentration of 2 mg/20 ml. Duplicate flasks were used for each amino acid ond incubation temperature was 25°C.

The lack of response to alanine war particularly noted in view of the response to the other neutral amino acids. Growth on glycerol or ribose was poor for the mutants as well as for the wild types. The mutants grew slower than the wild types on glycerol or ribose media, irrespective of the amino acid supplement.

2. Growth of phen-1 mutants on leucine-supplemented medium (sucrose-Vogel medium). Quantitative measurements (mycelial dry weight) on shaken cultures show that the rote of growth in leucine medium is very sensitive to the presence of certain amino acids and vitamins. The mutant strains used were H6196 (FGSC#492) ond H3791 (FGSC#504). Some observations were checked with UAl19 (FGSC#1 167).

(a) Most noticeable is the stimulation of growth by isoleucine, valine and threonine at equimolar concentrations (1 µmole/ml) Isoleucine has been further tested in varying proportions to leucine ond it was found that it did not promote growth when alone or in the presence of very low levels of leucine. In many cases a combination of L-isoleucine and L-leucine (each of 1 µmole/ml) gave growth several times higher than on 2 µmole/ml.

(b) Arginine, lysine, histidine ond methionine inhibit the rote of growth on leucine but it is not known whether the amount of growth at the stationary phase is affected or not. The kinetic and non-competitive features of inhibition by arginine hove been previously mentioned (Jho 1965 Neurospora Newsl. 7: 15). The inhibitory effect of basic amino acids war noted by Barratt and Ogata (1954) who first described phen-1 mutants. Most, but not all, phen-I isolates from crosses to wild types appear to be inhibited by arginine.

(c) Growth on leucine-supplemented medium is also reduced, at least in the "log" phse, by pyridoxine HCl (10mg/l) or thiamine HCl (10mg/l). Adenine and cytosine but not uracil (at concentrations of 1 µmole/ml) also appear to reduce the rate of growth in leucine medium.

3. Lack of response of phen-1 strains to alpha-keto precursors of aromatic amino ocidr ond leucine. Generally alpha-keto precursors are poor growth-promoters (sucrose Vogel medium) even for leaky phen-1 strains such as UA1 19. The idea that the poor response to on alpha-keto analogue might be on inherent characteristic due to the lesion in the phen-I locus has been tested (Jho 1968 Proc. Austral. Biochem. Soc. 1968: 8 (Abstr.)) as described below.

Crosses of two independent phen- 1 strains phen- 1 (UA 1 19); nos (89601) (FGSC#1 167) and phen-1 (H3791) (FGSC#504) were mode to two phen-2 stocks (E5212) given kindly by Dr. D. E. A. Catcheside. The progeny were classified into phen-I, phen-I; phen-2, and phen-2 on the basis of tests on minimol, minimal + leucine, minimal + phenylolonine media and linkage to the mating-type locus (phen-1 is very closely linked to mating-type).

The auxotrophic isolates or well as the parents were tested by conidial inoculations in liquid minimal, minimal + phenylolonine (2 µmole/ml) and minimal + phenylpyruvate (2 µmole/ml) on a shaker at 25°C. Visual examination showed that whereas all phen-2 isolates showed appreciable growth on phenylpyruvate medium according to expectations (Brockman et al. 1959 Arch. Biochem. Biophys. 84: 455), none of the phen-1 isolates grew on this medium when compared to minimal medium. Quantitative measurements were mode on some phen-1 isolates to check the visual observations. Thus, it appears that the phen-1 strains ore unable to take up, or utilize internally, phenylpyruvate for growth. Alternatively, these observations indicate that the NH2 group is essential for the growth-promoting activity of amino ocidr.

Dialyzed extracts of freeze-dried mycelia of a phen-I and a phen-2 strain were found to possess an isoleucine-phenylpyruvate aminotransferase activity. Specific activities in various conditions of growth were not measured but might be instructive. In vivo phen-1 iroloter did not grow on Vogel-sucrose medium supplemented with both isoleucine ond phenylpyruvate. - - - Research School of Biological Sciences, Australian National University, Canberra, Australia.