Jha, K. K. Notes on phen-l mutants.

1. Viability of conidia of phen-l mutants on different growth-promoting compounds.

phen-l mutants grow on minimal medium supplemented with any one of the aromatic amino acids or leucine or ethyl acetoacetate (Barratt and Ogata 1954 Am. J. Botany 41:763). In the course of attempts to estimate the reversion frequencies in three phen-l strains (H6196, H3791 and UA119), it was found that the conidia of these strains were not equally viable on sorbose-minimal agar medium supplemented with various growth-promoting compounds. The viability of conidia is approximately the same on L-tryptophan or L-tyrosine-supplemented medium and is about two times higher than that obtained in the presence of L-phenylalanine, L-leucine or ethyl acetoacetate. The three strains give similar results in this respect.

The results obtained with strain H6196 are illustrated in Figure 1. Note that the variations in the concentrations of a particular supplement do not alter the viability of conidia, except in the case of ethyl acetoacetate, which is a volatile compound. Note also that a combination of amino acids does not increase viability over that obtained with tryptophan or tyrosine alone, indicating that the population of conidia is not heterogeneous in this respect.

When compared with other compounds, L-tyrosine causes a more rapid development of colonies. Even in the presence of tryptophan or tyrosine, only approximately 50% of the conidia give rise to colonies. The effects of sorbose, variations in the age of conidia or the frequency of uninuclear conidia on the expression of viability are not known. We use Vogel's medium with 1% sorbose (autoclaved separately) and 0.1% glucose and the viability was determined with six-day-old conidia. It is possible that the differences in the viability of conidia may be partially responsible for the variations in the growth of phen-l strains in response to different supplements.

2. Frequency of phenotypic reversions to prototrophy in phen-l strains.

Recently the author discovered a new phen-l strain UA119 (FGSC#1167) among a group of tryptophan requiring mutants. The characteristic which principally drew attention to the strain was the extremely high frequency of phenotypic reversions. Some experiments were done to compare the "reversion" frequency of this strain with the frequencies in H6196 and H3791. The results are given in Table I.

Estimates of "reversion" frequencies are arbitrary in certain respects. On minimal-sorbose agar, the conidia of the three phen-l strains germinate and give rise to minute colonies which in general do not exceed one mm. in diameter after incubation for three days at 30°C. Counts were made of all colonies which exceeded the general size and were classified into three categories: small (1-2mm.), medium (2-4mm.) and large (4mm. or larger). Table I gives only the counts of colonies 2 mm. or larger, with the assumption that a genetic change is responsible for the increased growth. Variations in the morphology of the "revertant" colonies were also noticeable. The proportion of "large" colonies is approximately one in ten among the so-called "revertants". Since conidia give rise to minute colonies and presumably the number of nuclei increases with the increase in incubation period, the frequency of "reversions" has been found to be two to three times higher after seven days as compared to the frequency after three days.

It may be assumed that mutations at the phen-l locus, as well as at sites elsewhere in the genome, bring about prototrophy and the possibility that the large, fast-growing colonies are due to alterations at the phen-l locus is being tested.

3. Altered nutritional characteristics of some ascospore isolates from crosses of UA119 with H6196 and H3791.

Newmeyer (1963 NN#4:10) reported the frequent occurrence of "slow" phen-l isolates which do not respond well to phenylalanine-supplemented medium containing sucrose as a carbon source. The difference between "slow" and "fast" isolates was removed on glycerol medium.

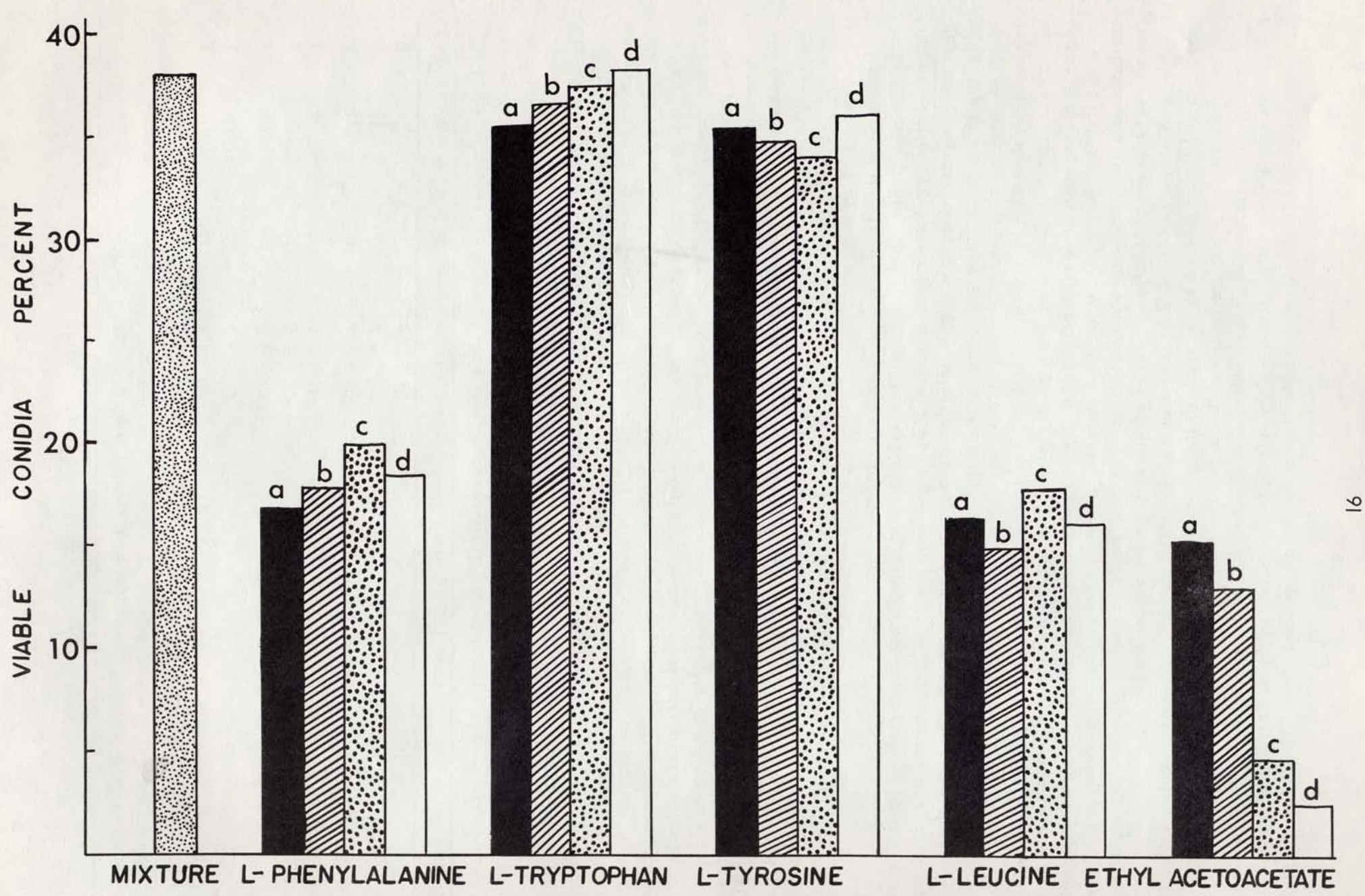

Fig. 1. Viability of conidia of the phen-1 strain UA119 on sorbose-agar media containing various levels of nutrients, singly or in combination. Letters a, b, c, d, indicate 1.5, 1.0, 0.5, and 0.25 micromoles per ml. of medium, respectively. The mixture contained 0.25 micromoles of each amino acid per ml.

TABLE I

Frequency of "reversion" in phen-1 strains.

Expt.	Mutant strain	Viability (%)	No. of viable conidia	No. of revertant colonies	Frequency of reversion per 10^6 viable conidia
1	UA119	61	6.1×10^6	617	101
2	UA119	41	4.1×10^6	416	101
3	UA119	44	4.4×10^5	73	166
4	H6196	50	5.0×10^6	13	2.6
5	H6196	38	3.8×10^6	28	7.4
6	H6196	50	5.0×10^6	23	4.6
7	H3791	48	4.8×10^6	21	4.5
8	H3791	45	4.5×10^6	208	46.0
9	H3791	62	6.2×10^6	73	11.8

(10^7 conidia in 1000 ml. medium spread over 70-90 plates except in Exp. 3 where 10^6 conidia on 10 plates)

A large number of ascospores from crosses between UA119 and two other phen-1 strains (H6196 and H3791) have been tested for response to media containing different growth supplements. Isolates which do not behave like the parental strains in their nutritional characteristics were found to be frequent.

Among 92 isolates, presumably H6196 derivatives (mating type a), in one cross between H6196 and UA119, three were found to grow slowly on minimal medium and none of the supplements had any effect on growth; three strains did not grow on phenylalanine; three isolates grew slowly on leucine and ethyl acetoacetate. The most interesting were eight "leucine-specific" isolates which grew only on leucine medium, whether the carbon source was sucrose or glycerol. These isolates did not grow on any of the aromatic amino acids, supplied individually or in combination, and they did not grow on ethyl acetoacetate medium. The gene and physiological basis of these "leucine-specific" phen-1 strains is under investigation.

In a second cross between UA119 and H6196, a total of 194 random ascospores were tested and only one "leucine-specific" strain was found. There were, however, many isolates which showed variations in response when compared with the parental strain. One isolate, for example, did not show any response to ethyl acetoacetate. A total of 277 random ascospores from two independent crosses between UA119 and H3791 were tested for "modified" phen-1 isolates. While other types of variants were present, no "leucine-specific" strains were found in these samples.

4. Inhibition of growth of the phen-1 strains by the basic amino acids.

Barratt and Ogata (Ibid.) reported that arginine and lysine produced a marked inhibition of growth when the phen-1 strain H6196 was grown in a medium supplemented with a limiting amount of phenylalanine. Six other amino acids produced a slight inhibition at the concentration tested. We have also noticed the strong inhibitory effect of arginine and lysine during growth tests and the crosses of phen-1 strain UA119 with arginine or lysine-requiring marker strains. Brockman, DeBusk and Wagner (1959 Arch. Biochem. Biophys. 84: 455) and Brockman (1964 J. gen. Microbiol. 34: 31) have shown that the inhibition of certain aromatic amino acid mutant strains due to the presence of some other amino acids in the medium can be explained by competition between the amino acids for a common permeability system. In their studies arginine and lysine were not found to be inhibitory for these mutants. phen-1 strains, on the other hand, are inhibited by the basic amino acids (including ornithine, which has been found to be inhibitory in the present study), irrespective of the presence of leucine or phenylalanine as a growth supplement.

Since arg-1 and arg-3 could be useful as closely linked markers for genetic studies with phen-1 strains, there is a utilitarian interest in the study of the phenomenon of inhibition. Some of the results obtained so far, using the strain H6196, are presented below:

(1) Arginine exerts an inhibitory effect at a relatively low concentration, particularly when leucine is the growth supplement. Almost maximal inhibition is exerted by arginine at a concentration of 0.15 μM/ml and further increase in the arginine concentration has no pronounced effect. The saturating concentration

may, however, be correlated to the amount of conidia.

(2) Variation of leucine concentration between 2-8 μM/ml does not alter the amount of growth obtained in the presence of arginine, indicating that the inhibition by arginine is non-competitive in nature.

Control (L-leucine) shaken cultures grow exponentially and the addition of arginine changes the growth curve to the arithmetic scale. It is not certain yet whether the "linear" rate of growth is assumed immediately after addition of arginine. An experiment in which arginine (0.2 μM/ml) was added to a shaken culture (100 ml. medium in 500 ml. Erlenmeyer flask; L-leucine 2 μM/ml.) six hours after inoculation, showed that the increase in dry mass could continue at the control rate for at least seven hours (approximate doubling time 210 min.). In another experiment in which arginine was added at the time of inoculation of conidia, a definite divergence from the control growth curve could be seen only after 9 hrs. of growth. In the same experiment observations of cultures older than 23 hrs. revealed that the arginine-treated cultures had resumed logarithmic growth. An analysis of the uptake of leucine in the presence of arginine and its relation to growth is in progress.

(4) The initial results indicate that arginine, in any concentration, does not inhibit growth when L-tryptophan or ethyl acetoacetate are the growth supplement. Growth at lower concentrations (near 0.25 μM/ml.) of L-tyrosine is inhibited by arginine and tests are under way to see if inhibition takes place at higher concentrations of tyrosine and various levels of arginine. If L-tryptophan is present at a growth-inhibitory concentration, the presence of arginine stimulates growth.

A similarity may exist between the mechanisms of growth inhibition of histidine-requiring mutants and of phen-I mutants in the presence of arginine and certain other amino acids. Mathieson and Catcheside (1955 J. gen. Microbiol. 13:72) have shown a correlation between the uptake of histidine and inhibition of histidine mutants in the presence of arginine and methionine or tryptophan. Their data with regard to the effect of increasing concentrations of arginine on the growth of a histidine-requiring strain are very similar to the effect of arginine on the growth of phen-I strains on leucine or phenylalanine-supplemented media. Their data also show that the effect of methionine alone on growth was different in its nature from that of arginine and that the response to inhibitors was not a consequence of the histidine requirement but was dependent on the genotype.

This work was supported by grants to J. Weijer from the National Research Council of Canada. - - -
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