DeBusk, B. G. ond A. G. DeBusk. The free amino acid pool of Neurospora.

The intracellular free amino acid pool of Neurospora hos beer, studied using a Beckman 1.20-E amino acid analyzer. A number of extraction methods were tried. It was found that hot water ond cold 5% TCA gave equally good results ond since the hot water method is much simpler, it hor been used throughout these studies.

Wild type Neurospora crassa 74-OR23-1A (FGSC#987) was grown on Vogel's medium N with 2% agar at 25°C for 5 days. The conidio were harvested and filtered to remove mycelial fragments. An aliquot of the resulting suspension was dried at 55°C and the volume of the suspension was adjusted to obtain a concentration of 10 mg (dry weigh+) conidio per ml. The samples were placed in a boiling water both for 20-30 minutes, centrifuged to remove the conidin, and the pH adjusted to 2.2 with any necessory volume adjustments. The some Procedure was followed with mycelio, except agar was omitted from the medium and the growth period was reduced to 3 days. Dry weight was obtained on a Portion of the pod. It was found that dry weight is about 14.5% of the squeezed wet weight. Protein content was determined by hydrolyzing extracted conidio (or mycelia) with 6 N HCl for 20 hours at 95°C, removing the HCl, filtering, ond adjusting the volume and pH. Separate determinations were necessary for tryptophan and cystine. Tryptophan values were obtained by hydrolyzing in 1 N NaOH at 95°C for 20 hours. Cystine was determined by treating a portion of the acid-hydrolyzate with H202 in the cold overnight and heating to remove the H_2O_2. This treatment results in the formation of some cysteic acid which is reported as cystine in the table.

Table 1. Amino acid content of pool ond protein.

Amino acid	Conidia			Mycelia		
	Pool*	% of total pool**	Protein'	Pool*	% of total pool**	Protein*
Tryptophan	2.5	0.8	15	0.4	0.1	33
Lyrine	5.0	1.6	171	38.9	5.4	260
Histidine	7.0	2.2	53	16.0	2.2	97
Arginine	8.5	2.7	115	47.5	6.6	212
Asportic acid	19.8	6.3	284	22.6	3.1	436
Threonine	11.2	3.6	139	33.9	4.7	217
Serine	59.4	19.0	162	107.4	14.9	273
Glutamic acid	82.8	26.5	249	103.3	14.3	401
Proline	1.0	0.3	123	17.8	2.5	115
Glycine	6.2	2.0	209	35.8	5.0	350
Alonine	81.0	26.0	235	183.1	25.4	417
Cystine	4.1	1.3	17	28.6	3.6	62
Valine	10.0	3.2	149	29.1	4.0	174
Methionine	5.5	1.8	6	16.2	2.2	65
Isoleucine	2.1	0.7	109	9.4	1.3	120
Leucine	2.9	0.9	186	16.2	2.2	297
Tymsine	1.5	0.5	60	6.9	1.0	94
Phenylalanine	1.5	0.5	81	7.2	1.0	137
Hydroxyproline			58			28
X-I (unidentified)				3.6	.5	71
Total	312.0		2186	723.9		3859

*Expressed as µMoles/g dry weight. **Expressed in terms of mM.

The free amino acid pool was found to be consistent and stable. The pool was not decreased by dialysis or starvation (omission of either carbon-source, nitrogen-source, or both) with the exception of a slight decrease of glutamic acid and, to a lesser extent, alanine. When the growth medium was supplemented with some 15 different amino acids, either singly or together, no difference in the free amino acid content of the conidio was found.

If the conidia ore incubated in Vogel's medium N supplemented with on amino acid (except glutamic ond aspartic acids), on increase in the pool con be seen, in some cases a many-fold increase (e.g., phenylalanine increases from l-2 µM/g to 100 µM/g). However, with the exception of orginine, as soon as sucrose is added to the incubation medium or the exogenous amino acid is removed, the pool concentration drops. By careful selection of the amino acids (i.e., members of different "transport families") and the conditions, as many as five amino can be increased at one time, although not maximally. Again, it was found that this "imbalance" is corrected when the conidio ore placed under growing conditions. If the conidia ore incubated in a mixture of all the amino acids present in the pool, even if they ore in the proper ratios, no change in the pool con be detected.

The amino acid pools from a number of different wild types, 1A, Em 5256A, Sy4f8a, ond nine geographic isolates were examined. Some strains hove pools which ore very similar, although not identical to 74A; others, particularly certain of the exotic strains, hove markedly different pools. The segregation of pool patterns in recombinants is as yet unknown.

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