

The free amino acid pool of Neurospora

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The free amino acid pool of *Neurospora*

Abstract

Free amino acid pool

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The intracellular free amino acid pool of *Neurospora* has been studied using a Beckman 1.20-E amino acid analyzer. A number of extraction methods were tried. It was found that hot water and cold 5% TCA gave equally good results and since the hot water method is much simpler, it has 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 conidia 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 weight) conidia per ml. The samples were placed in a boiling water bath for 20-30 minutes, centrifuged to remove the conidia, and the pH adjusted to 2.2 with any necessary volume adjustments. The same procedure was followed with mycelia, 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 conidia (or mycelia) with 6 N HCl for 20 hours at 95°C, removing the HCl, filtering, and 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 H₂O₂ in the cold overnight and heating to remove the H₂O₂. 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 and protein.

Amino acid	Conidia			Mycelia		
	Pool* % of total pool**	% of total Protein'	Pool* % of total pool**	Pool* % of total pool**	Protein*	
Tryptophan	2.5	0.8	15	0.4	0.1	33
Lysine	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
Aspartic 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
Alanine	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
Tyrosine	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-1 (unidentified)				3.6	.5	71
Total	312.0		2186	723.9		3859

*Expressed as $\mu\text{Moles/g}$ 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 conidia was found.

If the conidia are incubated in Vogel's medium N supplemented with an amino acid (except glutamic and aspartic acids), on increase in the pool can be seen, in some cases a many-fold increase (e.g., phenylalanine increases from 1-2 $\mu\text{M/g}$ to 100 $\mu\text{M/g}$). However, with the exception of arginine, 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 acids can be increased at one time, although not maximally. Again, it was found that this "imbalance" is corrected when the conidia are placed under growing conditions. If the conidia are incubated in a mixture of all the amino acids present in the pool, even if they are in the proper ratios, no change in the pool can be detected.

The amino acid pools from a number of different wild types, 1A, Em 5256A, Sy4fga, and nine geographic isolates were examined. Some strains have pools which are very similar, although not identical to 74A; others, particularly certain of the exotic strains, have markedly different pools. The segregation of pool patterns in recombinants is as yet unknown.

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