Johnson, H. N. Purification of Neurospora ß-galactosidase.

This communication will describe an efficient method for the purification of the smaller ß-galactosidase of Neurospora (pH 4.2 enzyme). Wild type Neurospora crassa (74-OR-A) was used for the isolation of the enzyme. Large quantities of mycelia were prepared by incubating conidia in ten liters of Vogel's minimal salts plus 0.7% L-arabinose and 0.3% sucrose. The culture was aerated by bubblingand incubated for 96 hours at 25°C. The culture was harvested by straining through cheese-cloth, washing with cold distilled water, and compressing the mycelial mat by vacuum filtration. Cells were disrupted for protein extraction in a Sorvall Omni-Mixer with 0.01 M potassium phmphote buffer, pH 7.5 (Buffer A) at a ratio of 6 ml of buffer/g of mycelia. This was followed by sonication with a Bronwill Biosonik at maximum probe intensity for 30 sec per 100 ml of homogenate. All procedures were carried out at 4°C. The homogenate was gently shaken for 2 hrs and then centrifuged at 27,000 x g for 20 min. The supernatant was used as the crude extract.

Table 1. Summary of purification.

step	Volume ml	Protein mg	Total activity enzyme units	Specific activity μ/mg	Recovery %
I. Crude extract	200	3,745	20,255	5.4	100
2. 75% $AmSO_4$ ppt	100	1,075	15,333	14.1	75
3. pH 4.0 soluble	100	178	15,333	86. 1	76
4. After dialysis	110	38	15,000	392	74
5. 75% $AmSO_4$ ppt after dialysis	20	6.6	13,775	2078	68
6. CM eluant	5	0.298	9,925	33,250	49

Table 2. Amino acid composition of the pH 4.2 ß-galactosidase of Neurospora 74A.

Amino acid	Residues/MW of 96,000 Found	Nearest integer
Lysine	80.77	81
Histidine	14.48	15
Arginine	0	0
Aspartic acid	84.58	85
Threonine	43. 18	43
Swine	202.06	202
Glutamic acid	140.84	141
Proline	26.04	26
Glycine	258.57	259
Alanine	92.33	92
Cystine	——	—
Valine	22.22	22
Methionine	1.27	1
Isoleucine	14.48	15
Leucine	26.04	26
Tyrosine	13.21	13
Phenylalanine	7.49	8
total		1,029

The crude extract is taken to 33% saturation with ammonium sulfate at pH 7.5 and stirred for 20 min. After centrifugation at 27,000 x g for 20 min., the supernatant is token to 75% saturation with ammonium sulfate at pH 5.0 and stirred for 4 hn. The precipitate is collected by centrifugation at 27,000 x g and dissolved in 1/10 the original volume of Buffer A. This solution is made pH 4.0 by the addition of 1 M citric acid and allowed to stand in the cold for 8 hn. The precipitate is centrifuged out and the supernatant is dialyzed against distilled water for 6 hrs. The dialyzed supernatant from centrifugation is taken to 75% saturation with ammonium sulfate at pH 5.0 and allowed to stand for 10-12 hrs. The precipitate is collected and dissolved in 1/100 the original volume of Buffer B (0.008 M Sodium phosphate-citrate, pH 4.2) with 0.01 M 2-mercaptoethanol. This solution is then chromatographed on a CM Sephadex column equilibrated with Buffer B plus mercaptoethanol. The enzyme is eluted with a NaCl gradient (0.008-1.0 M in Buffer B). The peak fractions contain an electrophoretically pure enzyme preparation. As seen in Table 1, the overall purification is some 6000-fold with a 49% recovery.

A sample of the purified enzyme was hydrolyzed in *b N* HCl and its amino acid composition war determined with a Beckman 1208 automatic amino acid analyzer. The amino acid composition is shown in Table 2. The number of residues was bored on a molecular weight of 96,000. The composition was determined on the basis of three individually purified sampler. The absence of arginine in this enzyme may be an important point in future work with this enzyme, especially with respect to peptide mapping.

This work supported in part by the NIH Training Grant in Genetics (T01-GM01316) to Florida State University ▪ ▪ ▪ Genetics Laboratories, Deportment of Biological Science, Florida State University, Tallahassee, Florida 32306.