Fungal Genetics Reports

Volume 14 Article 14

Determination of protein in conidia

G. Schneider

W. Klingmuller

W. Sebald

F. Kaudewitz

Determination of protein in conidia

Abstract
Determination of protein in conidia

Schneider, G., W. Klingmüller, W. Sebald and F. Kaudewitz. Protein determination in conidia of Neurospora crassa.

During experiments in which the rate of uptake of different sugars by conidia of a number of different strains of Neurospora crassa was to be estimated and referred to their protein content, a quick and reliable method for measuring the protein content of ungerminated conidio was needed. For technical reasons, we first tried to measure their protein content directly by applying a modification of the Biuret method as described by Beisenherz et al. (1953 Z. Naturforschung 8b: 555) to whole conidia.

Individual steps of this modification are as follows: (1) Prepare conidial suspensions of 5 x 10^7 conidia/ml. (2) Precipitate the protein in 1 ml of the conidial suspension with 0.2 ml 50% TCA, shake well and spin down. (3) Wash two times with a mixture of 3 parts ethyl alcohol and one part ethyl ether and a third time with ethyl ether to remove carotenoid color and other lipids. (4) Let the ether evaporate from the pellet and resuspend in 1 ml Biuret reagent. (5) Incubate for 30 min. at room temperature on a shaker. Centrifuge and transfer the supernatant info a 1 cm cuvette. (6) Read the absorption at 546 mμ. Add a small amount of KCN powder to the cuvette, stir and wait 1-2 min. until the remaining absorption is constant, and read again. The difference in absorption is due to the protein in the solution (A_p). (7) Measure the absorption of the Biuret reagent alone before and after addition of KCN. The difference in the blank absorption (Ab) is to be subtracted from A_p. (8) Refer A, — Ab to a calibration curve, obtained for bovine serum albumin to obtain mg protein/ ml.

Applying this procedure and increasing the incubation time at room temperature from 30 min. to 120 min., a marked increase of the resulting absorption was observed (Fig. 1, curve a). This finding was thought to indicate that not all of the protein in a whole conidium was available freely to the Biuret-reagent. This was confirmed by increasing the temperature of incubation up to 50°C, when a further increase in absorption was observed (Fig. 1, curve b). Neither elongation of incubation-time up to 120 min. nor increase of incubation-temperature up to 50°C hod a significant effect on absorption of calibration samples containing bovine serum albumin.

In additional experiments, conidia were therefor disrupted either by treating them 1 to 3 times with the X-press (AB Biox, Box 235, Nacka 2, Sweden) with a 25 ml volume operated at -25 to -35°C at ca. 2000 kg/sq cm, or by smashing them with glass beads (d = 0.45-0.50 mm) for 5 to IO minutes in a homogenizer

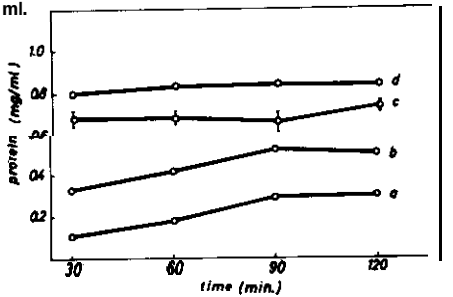

Fig. 1. Values for whole or disrupted conidia (5 x 10^7/ml) after incubation with the reagent for different periods of time.

(Vibrogen-Zellmühle, Fa. E. Bühler, Tübingen, Germany). The results obtained by there disintegration methods document a further increase in protein content of the supernatant (Fig. 1, curve c for 3 times X-pressing, curve d for gloss-bead disintegration). They demonstrate that in the broken cells either protein is available faster to the reagent or additional protein is set free to react with the reagent. Visual observation of the state of the cells by means of phase contrast microscopy showed less than 5% intact conidia after gloss-bead disintegration, but co. 50-25% after 1-3 X-pressings, respectively, a finding which may explain the fact that the latter procedure gives a lower protein value than the former. To obtain reliable data on the protein content of Neurospora conidio, the complete disruption of the cells is therefore an essential prerequisite.

We are grateful to Dipl. Biol. R. Schweyen and Miss D. Hacker for valuable technical suggestions. This work was supported by the Deutsche Forschungsgemeinschaft. Institut für Genetik, Universität München, München, Germany.