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R. B. Drysdale

M. Fling

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Abstract

Assay of proteolytic enzyme(s) from *Neurospora crassa*

Drysdale, R. B. and M. Fling. Assay of proteolytic enzymes from Neurospora crassa.

Proteolytic activity is measured by incubating samples of extract with casein, stopping the reaction by addition of perchloric acid and measuring the amount of tyrosine, both free and peptide, in the perchloric acid supernatant.

A. Preparation of extract: The mycelium is pressed dry on filter paper and then ground with a little sand in a cold mortar. Sodium phosphate buffer (0.1 $\bar{M}$; pH 6.0; 10 parts v/w) is added and the extract centrifuged at 10,000 x g for 10 minutes. The supernatant is decanted and used for the enzyme assays.

B. Incubation: The incubation mixture contains 0.9 ml phosphate buffer (pH 6.0, 0.1$\bar{M}$) containing 400 µg casein plus 0.1 ml extract. The casein in buffer is incubated at 35°C for 5 minutes and the extract is added. After a further 15 minutes at 35°C, 1 ml of 1$\bar{M}$ perchloric acid is added to stop the reaction. After 10 minutes at room temperature, the tubes ore centrifuged and the supernatant is pipetted off. A blankin which the perchloric acid is added before the extract is run far each extract used.

C. Tyrosine estimation: (Udenfriend and Cooper 1952 J. Biol. Chem. 196: 227):

Reagents: (1) 1-nitroso-2-naphthol solution; 0.1% nitrosonaphthol in 95% ethanol. (2) Nitric acid - sodium nitrite; 2.6 M nitric acid containing 0.05% sodium nitrite. This may be conveniently prepared by adding 0.5 ml of 2.5% sodium nitrite to 24.5 ml of 2.6M nitric acid immediately before use.

Assay: The reaction mixture contains 1 ml of supernatant + 0.5 ml nitrosonaphthol solution + 0.5 ml nitric acid-sodium nitrite. Mix and heat at 55°C for 30 minutes. Cool, add 5 ml 1,2-dichloroethane to each tube, mix thoroughly and centrifuge to break the emulsion. The dichloroethane layer contains the unreacted nitrosonaphthol. Measure the optical density of the upper layer at 450 mμ. Reagent blanks and tyrosine standards should be run with each set of assays. ■ ■ ■ Division of Biology, California Institute of Technology, Pasadena, California.