NUTRITION OF EIDAMELLA DEFLEXA. IV. RATE OF UTILIZATION OF SUGAR AND AMINO NITROGEN COMPARED WITH RATE OF GROWTH AND PIGMENT ELABORATION

by

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(with 2 figs.)

Three previous papers in this series (Kuehn & Crosby, 1960a, 1960b, 1960c) were directed toward the establishment of a synthetic medium for the growth of Eidamella deflexa (Berk.) Benjamin in shaken flasks. The nature of the nitrogen source proved to affect the response of the fungus to a given carbohydrate, and the reciprocal condition was also found to exist. It was learned that glycine was the best defined nitrogen source for growth, and that sorbitol, lactose and maltose were the best carbohydrates for growth with glycine as the nitrogen source. Various complex nitrogenous materials were capable of supporting excellent growth, and these included yeast extract, corn steep liquor and peptone. The relation of the medium to the elaboration of a water-soluble red pigment by this fungus was also studied, and, in general, it was found that the medium optimum for growth was also optimum for pigmentation. Studies regarding chemical isolation and identification of this red pigment are still in progress.

A medium was established which thus far was considered to be optimum. The composition of this medium is as follows: 5% glycine, 5% maltose, 0.1mg% thiamin, 0.2 mg% biotin and 8 mg% uracil with a pH of 5.0.

The purpose of the present investigation was to correlate the rate of growth and pigment elaboration with the rate of utilization of nitrogen and carbon. In addition, the maltase and proteolytic activity of the culture filtrates were investigated.

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The strain of *Eidamella deflexa* which was used was the same employed in previous work (Kuehn & Crosby, 1960a, 1960b, 1960c) and is designated as RSA (Rancho Santa Ana Botanic Garden) 57. Stock cultures of the mold were maintained on YpsS medium: 1.5% soluble starch, 0.4% yeast extract, 0.1% K$_2$HPO$_4$ and 0.05% MgSO$_4$·7H$_2$O. After such stock cultures had developed a good supply of ascospores they were stored at 4°C. Inoculum for the experimental flasks was brought up in the following manner. Ascospores and mycelium were transferred by means of an inoculating needle from the stock slant to 100 ml of liquid medium contained in 500 ml Erlenmeyer flasks. This inoculum medium had the following constitution: 1% peptone, 4% sucrose, pH 7.0. The inoculum flasks were incubated on a reciprocal shaker for 6-12 days prior to their use.

The test flasks contained 100 ml medium per 500 ml Erlenmeyer flask, and were inoculated with 3% nine-day-old submerged growth from the inoculum flasks. Two tests media were used, with the following composition: (1) 1.5% glycine, 4% maltose, 0.1 mg% thiamin, 0.2 mg% biotin, 8 mg% uracil, 0.2% MgSO$_4$·7H$_2$O and 0.2% K$_2$HPO$_4$ at pH 7.0; (2) 4% sucrose, 1% peptone at pH 7.0. The concentrations of the inorganic constituents of the medium were selected on the basis of preliminary investigations regarding the inorganic requirements of this fungus, which will be reported upon in a separate publication. The experimental flasks were incubated at 28°C on a reciprocal shaker. At various intervals of time during the incubation period replicate pairs of flasks were removed from the shaker. The mycelium was separated from the culture broth by centrifugation and was measured on a dry weight basis in accordance with the procedure explained in detail previously (Kuehn & Crosby, 1959). The culture filtrate was then assayed for pigment, residual reducing sugars and residual non-protein (amino) nitrogen. To measure pigment production an aliquot of the culture filtrate was diluted to a suitable degree and the optical density was measured against a water blank in optically clear 18 x 150 mm test tubes using a Bausch and Lomb Spectronic 20 colorimeter at a wave length of 450 m.$\mu$. The dilution of culture filtrates was 1:100.

Residual non-protein nitrogen was measured using a micro-kjeldahl method for the determination of nitrogen. The precise procedure was as follows. The volume of the culture filtrate from each flask was measured exactly. Five ml of 0.3 N trichloracetic acid was added to a 5 ml aliquot of culture filtrate. The mixture was placed in a boiling water bath for 5 minutes to complete removal of protein. After cooling, the material was centrifuged to remove proteinaceous material. A 2 ml aliquot was digested using a standard micro-kjeldahl procedure to obtain the total nitrogen value. A second 2 ml aliquot was distilled to determine the amount of ammonia nitrogen.