

## PRODUCTION OF INDOLEACETIC ACID BY *CEPHALIOPHORA TROPICA*

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ABSTRACT. — Mycelium of *Cephalio-phora tropica* produced indoleacetic acid (IAA) in liquid medium when grown in either continuous light or dark. Fresh weight of light-grown mycelium was about twice that of the dark-grown mycelium, while dry matter produced in both treatments was about the same. Medium of dark-grown mycelium contained more IAA than that of light-grown.

Many fungi are known to produce indoleacetic acid (IAA) (Gruen, 1959), and inhibition of their growth by this substance is common (Cochrane, 1958). Some fungi produce IAA in the absence of an added precursor in the medium (Ulrich, 1960) although IAA synthesis usually requires the addition of tryptophan or a metabolically related compound (Bailey and Gentile, 1962; Brian, 1957; Mace, 1965; Thimann, 1955; and Wolf, 1952). Gentile and Klein (1955) suggested that *Diplodia natalensis* Pole-Evans synthesized IAA in medium with or without tryptophan indicating the possibility that this fungus was autotrophic for this substance. They reported that IAA in the growth medium could reach inhibitory levels through this synthetic activity. Bailey and Gentile (1962) separated and identified several indole compounds synthesized by *D. natalensis*. Using C<sup>14</sup>-labeled tryptophan they demonstrated that tryptophan proceeded to indoleacetic acid, indolelactic acid, and tryptophol. The fungus also converted tryptamine to indoleacetic acid.

Weik and Pappelis (1964) demonstrated that *Cephalio-phora tropica* Thaxter grew well on many nitrogen sources but poorly in a medium with tryptophan as the nitrogen source. Since tryptophan normally is considered to be a poor source of nitrogen for fungi (Cochrane, 1958) it was hypothesized that *C. tropica*

may be autotrophic for IAA and that the addition of tryptophan to the growth medium may be permitting the fungus to convert this to inhibitory levels of IAA. This paper presents data on a study of the production of IAA by *C. tropica* grown in continuous light or dark.

### MATERIALS AND METHODS

A small piece of *Cephalio-phora tropica* obtained from the leading edge of a colony growing on potato dextrose agar was grown in the dark in 50 ml of autoclave-sterilized Czapek-Dox broth medium (Fisher Scientific) containing 0.5 g of Yeast Extract (Baltimore Biological Laboratories) per liter on a rotary shaker at 110 rpm in 125 ml Erlenmeyer flasks. The colony was isolated, washed, and macerated at high speed as described by Weik and Pappelis (1964). One ml of the macerated mycelium was used to inoculate additional flasks containing 50 ml of sterile Czapek-Dox broth medium containing 0.5 g of BBL Yeast Extract and 0.5 g of vitamin free caseamino acids (Difco Laboratories) per liter. Flasks were divided into two groups. Flasks of one group were wrapped with aluminum foil to cover all except the cotton plug. The cotton plug was covered with a 5 inch square of foil and the four corners pushed downward to make a loose cap. The second group was covered only with the aluminum caps. Both groups were placed on a rotary shaker illuminated (300 foot-candles) with Gro-Lux fluorescent tubes (Pappelis, Schmid and Koike, 1964) and agitated for 4 days at 110 rpm. At the end of the growth period, the mycelia from 5 flasks were harvested, washed quickly with distilled water, and collected with a Buchner funnel on a filtering flask using a uniform 40 psi water pressure provided by a Buchler Water-

Booster pump (aspiration for a three minute interval). The mycelial pads were weighed, dried at 70°C for 24 hours, cooled in a desiccator and reweighed.

The culture filtrates were collected and extracted for IAA according to the methods described by Sequeira and Kelman (1962). The concentrated material was chromatographed with isopropanol-ammonium hydroxide-water (10:1:1), *n*-butanol-ammonium hydroxide-water (100:3:18), and *n*-butanol-ethanol-water (4:1:1). IAA was detected on paper using U.V. fluorescence and color reaction with the modified Salkowski reagent (Gordon and Weber, 1951).

#### RESULTS AND DISCUSSION

The growth of *C. tropica* in light and dark differed in that the fresh weight of mycelium grown in continuous light was 2.12 g total in the five flasks while that for mycelium grown in the dark was 1.03 g. The mycelial dry matter produced in the light was 0.47 g total in the five flasks, and 0.43 g of dry matter was produced in the dark. These differences in dry weights were not considered to be significant. The mycelium in the light and dark treatments appeared similar in color and texture, and no significant viscosity differences in growth media were detected.

The presence of IAA in the culture filtrate was indicated chromatographically by comparison with known IAA in three different solvent systems. The chromogenic spray reaction with the modified Salkowski reagent was more sensitive in detecting the presence of IAA than U.V. fluorescence. More IAA was present in filtrates of dark-grown mycelium. Thus, mycelia of *C. tropica* grown in the light had twice the fresh weight, the same dry weight, and a decreased amount of IAA as compared to that of mycelia grown in the dark.

Whether light affected the synthesis or destruction of IAA was not determined. Since enzymic IAA oxidation often has been shown to be light-dependent (Hare, 1964), the lower amount of IAA in light-grown filtrates could be explained if the fungus produces an IAA oxidase.

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