THE EFFECT OF LIGHT ON THE AMINO ACID METABOLISM OF *DIPLODIA ZEAE*

DIANE TEGTMEIER and A. J. PAPPELIS
Southern Illinois University, Carbondale

ABSTRACT.—Mycelial extracts and culture media from *D. zeae* grown simultaneously on a rotary shaker in the light and dark were assayed for qualitative amino acid content. A distinct difference was found in amino acid content of media from light-grown and dark-grown cultures, with very little difference in the mycelium.

Several studies have been conducted concerning the amino acid metabolism of fungi. Woolley and Peterson (1936) were the first to analyze the free amino acids found in the mycelium. Since that time, other workers have studied amino acid content in spores, mycelial hydrolysates and extracts, and also in culture media (Broyles, 1952; Murray and Zschiele, 1956; DeVay, 1952, 1954; and Jones, 1963). Pillai and Srinivason (1956), working with *Aspergillus flavus*, investigated the free amino acid content of mycelium, acid hydrolysate of mycelial residues, and the released free amino acids in the culture medium over a 30 day period of time. The effect of light on the amino acid metabolism has not been considered by any of these investigators.

A recent review of photobiology of fungi has been presented by Carlile (1965). Several areas of photobiology were discussed. Work with the watermold *Blastocladiella emersoni* by Cantino and coworkers showed an increased growth rate in illuminated cultures in contrast to unilluminated cultures. This increased rate of growth was accompanied by an increase in carbon dioxide fixation and polysaccharide synthesis. In addition, a definite effect of light on various enzymes produced by the fungus that are involved in a succinate-α-ketoglutarate-isocitrate cycle, nucleic acid biosynthesis, and arginine metabolism was demonstrated. Growth of another watermold, *Thraustochytrium roseum*, has been reported by Goldstein (1963) to be stimulated by light.

The limited work with light effects on filamentous fungi was also reviewed by Carlile. The growth rate of *Sclerotina fructigena* on malt agar is much higher in the light, while growth of *Penicillium clavigerum* on Czapek agar was depressed by light. The response to light differed in various types of medium. Carlile suggests that under optimal growth conditions the light and dark metabolism of many species is equally effective, but that under suboptimal growth conditions the fungi are more subject to metabolic changes induced by light or dark conditions. Katsanos and Pappelis, in unpublished work in this laboratory, have found a two-fold increase in fresh weight in light-grown cultures of *D. zeae* over dark-grown cultures.

Light effects on sporulation also have been reported. Kaiser (1964), working with *Verticillium albo-atrum*, observed a blue light inhibition of microsclerotium formation. Whitney (1964) and Robbins and Hervey (1960) reported light inhibition of basidia formation in *Rhizoctonia solani* and *Poria ambigua*, respectively.

The biochemical processes effected by light in these filamentous fungi have not been elucidated. In the present work, the effect of light on amino acid synthesis and their release into the culture medium by *Diplodia zeae* (Schw.) Lev. over a period of time was investigated. It is a preliminary attempt to more carefully determine the effect of light on *D. zeae* and its possible effect on pathogenicity in roots or stalks of corn.

Methods and Materials

Inoculum for this study was prepared by transferring a piece of mycelium with underlying agar (5 mm approximate diameter) from a potato dextrose agar plate into a 125 ml Erlenmeyer flask containing 50 ml of Czapek-dox broth medium (pH 4.5 after autoclaving). After a 5 day incubation period, the liquid medium was decanted and the mycelium fragmented for 1.5 minutes in a sterile, semi-micro Waring Blendor cup containing 50 ml of sterile, distilled water. Three ml of this suspension was transferred to several 125 ml Erlenmeyer flasks each containing 50 ml of Czapek-dox medium. One half of the flasks were wrapped with aluminum foil for the dark study. All flasks were placed on an illuminated rotary shaker (Pappelis, et al., 1964) operating at 105 r.p.m. at room temperature (25 - 28°C). Three flasks each of mycelium grown in the light and in the dark were removed for amino acid extraction and determination at 2, 4, 8, and 10 days of incubation.

The cultures were filtered separately on Buchner funnels with reduced pressure. Filtrates were removed for study before the mycelium was washed. Three volumes of 95% ethanol were added to the syrup-like filtrate and heated to boiling to precipitate the proteins. This mixture was transferred to centrifuge bottles and spun at 3,000 r.p.m. The supernatant was brought down to a volume of 10 ml using a rotary flash evaporator. Further purification of the medium was found to be necessary since carbohydrates interfered with the amino acid separation on the paper chromatograms. The ion-exchange method of purification described by Thompson, Morris and Gering (1959) was used. The eluates from the columns were evaporated to a 2 ml volume.

The mycelial mats obtained from filtration were quickly washed with 50 ml of normal saline and refiltered. This was done three times. The washed mycelial mats were transferred to a glass mortar and homogenized in 80% ethanol. After heating to boiling, the extract was cooled and centrifuged at 3,000 r.p.m. The supernatant was evaporated to dryness, and the residue made up to a volume of 2 ml with 10% ethanol. Ion exchange purification was not necessary since no interference of separation was observed on paper chromatograms.

Two-directional, descending paper chromatography of 25 lambda of extract using 23″ x 23″ Whatman #3 paper was employed to separate the amino acids. Phenol (Chromatography grade, liquefied): water (4:1 v/v) was used as the first-direction solvent; butanol: acetic acid: water (9:1:2.5 v/v/v) as the second-direction solvent. The first solvent was allowed to run for 24 hours, and the papers were dried at room temperature (25 - 28°C) for another 24 hours. The second direction also was run for 24 hours and dried 5 to 10 hours.

The spray reagent used, a modification by Ramon Tate (unpublished) of the Moffat and Lytle (1959) color reagent, formed various shades of reds, blues and tans characteristic for each amino acid. Two stock solutions were prepared consisting of (1) 1% (w/v) cupric nitrate in acetone and (2) 0.2% w/v ninhydrin and 1% (v/v) acetic acid in acetone. Immediately before spraying the 1% cupric nitrate was diluted to 0.03% with acetone and mixed with an equal volume of the ninhydrin stock solution. The papers were dried and the colors developed by heating. The R_f values and colors were compared to known standards determined using the above procedures singly and in mixtures.

Results

The free amino acid content of the mycelium and of the culture media is presented in Table 1. Qualitatively, the amino acids were essentially the same in the mycelium of light-grown and dark-grown cultures, except for the later appearance of lysine in the mycelium grown in the dark. A similar, unidentified spot was found on chromatograms of both extracts at 4, 8, and 10 days. Also, one more unidentified ninhydrin spot was present on the chromatograms of the dark culture at 4, 8, and 10 days.

Analysis of the culture media revealed a distinct difference in the amount of

Table 1. — Free amino acids present in the culture media and mycelial extracts of *D. zeae* grown in the light and in the dark at 2, 4, 8, and 10 days of incubation.

Amino Acid	Mycelial Extract								Culture Media							
	Light				Dark				Light				Dark			
	2	4	8	10	2	4	8	10	2	4	8	10	2	4	8	10
α alanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
arginine											+	+				
asparagine		+	+	+		+	+	+			+	+			+	+
cysteine-cystine		+	+	+		+	+	+								
glutamine											+	+				
glutamic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
glycine		+	+	+		+	+	+	+	+	+		+			
histidine	+	+	+	+	+	+	+	+		+	+	+				
leucine-isoleucine		+	+	+		+	+	+			+	+				
lysine	+	+	+	+		+	+	+								
methionine		+	+	+		+	+	+		+	+	+				
proline		+	+	+		+	+	+			+	+				
serine	+	+	+	+		+	+	+					+			
threonine		+	+	+		+	+	+			+	+				
tryptophan												+				
tyrosine		+	+	+		+	+	+								
valine		+	+	+		+	+	+								

amino acids released into the medium under the different light conditions. In the light-grown cultures, nearly all of the amino acids found in the mycelium were gradually released into the medium with the exception of cysteine-cystine, serine, tyrosine, and valine. Arginine, glutamine and tryptophan were found in the medium but not in the mycelium. In the dark-grown cultures, only α-alanine, asparagine and glutamic acid accumulated in the medium at 10 days. Glycine and serine appeared only in the two-day culture; serine never appeared in medium from light-grown cultures. The total accumulation at 10 days in the light was 12 amino acids and in the dark only 3.

Discussion

When the data from this study are compared with those of Pillai and Srinivason (1956), similarities are noted in that the mycelium contains more free amino acids than does the culture medium. However, it is not advisable to compare differences between the data regarding free amino acid content in the culture medium of light and drak grown mycelium to that grown under laboratory conditions where alternating light and dark occurs, since, as our data have shown, the difference is considerable. In future studies directed to free amino acid or protein content in the mycelium or the cultural medium, light should be regarded as an important variable requiring control.

Whether the difference observed in amino acid release into the culture medium is due to an alteration of protein synthesis thereby altering the requirements of the amino acid pool, or due to a stimulation of amino acid biosynthesis is yet to be investigated.

The various effects of different wavelengths of light also are to be considered. Kaiser (1964) observed microsclerotia formation at several wavelengths and noted distinct differences not only in the microsclerotia, but also in pigmentation. An investigation of this sort would be a further aid in elucidation of the mechanisms of light action on amino acid synthesis and release by *D. zeae*.

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