EFFECT OF LIGHT AND MEDIA UPON GROWTH AND MELANIN FORMATION IN CLADOSPORIUM MANSONI

by

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(with 3 figs.)

Cladosporium mansoni is a human pathogen that is highly pleomorphic and resembles *Aureobasidium* in the formation of yeast-like cells that often are dark in the aggregate. In fact, it has been stated that the organism with which *Aureobasidium* is most often confused is *Cladosporium* (COOKE, 1962). Therefore, recent studies of the carbon nutrition and formation of melanin in *Aureobasidium* (LINGAPPA, *et al.*, 1963) were extended to include *Cladosporium mansoni* in order that a comparison could be made of these aspects of the physiology of the two organisms.

MATERIALS AND METHODS

The cultures of *Cladosporium mansoni* used in this work were obtained from the American Type Culture Collection (ATC #762) and were maintained on potato dextrose and nutrient agars. Defined media #1 and #2, whose composition is provided in LINGAPPA, *et al.*, (1963), were used in nutritional experiments along with the *Neurospora* "minimal" medium (RYAN, 1950). All undefined media used were the product of the Difco Co., Detroit, Michigan.

Techniques for harvesting cells and determining growth are as described previously (LINGAPPA, *et al.*, 1963) as are the methods used in the extraction and analysis of melanin.

RESULTS

When *C. mansoni* is grown on defined medium #1, containing asparagine, the results provided in Table I are obtained. Trehalose is the best carbon source of those tried and sucrose, maltose, all of the monosaccharides and dextrine also support good growth. By contrast, starch and lactose support only sparse growth, whereas inulin and chitin are not used at all. Melanin, by the criteria used in the previous paper, is formed in cells grown on the good carbon sources, except for those on fructose. The most abundant pigmentation is found in cultures grown on trehalose. Furthermore, extracellular granules never are formed in any cultures grown on defined medium #1 with asparagine.

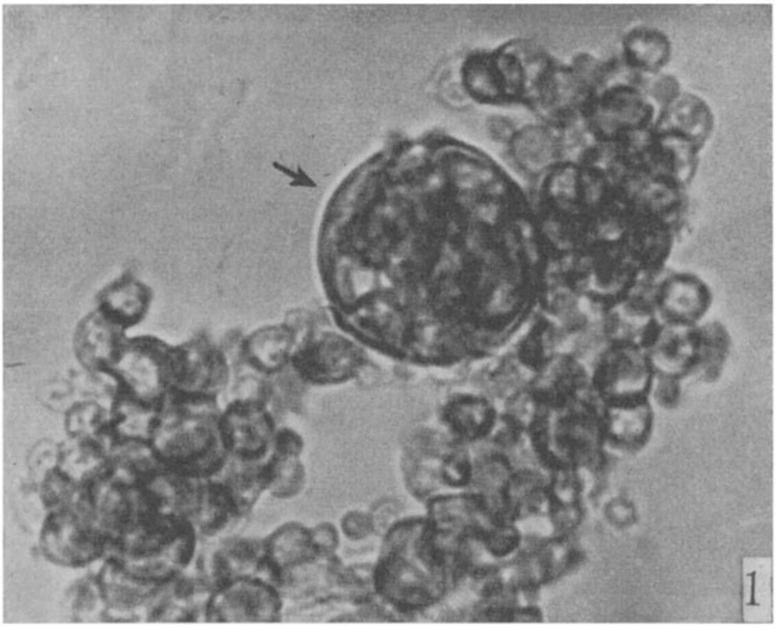

Fig. 1. Appearance of *Cladosporium mansoni* grown for two weeks in defined medium #2 in the dark. Arrow indicates a "giant" cell. Magnification — approx. 2,000×.

The pigment from cells of *Cladosporium* was extracted and absorption spectra obtained as shown in figure 3. Straight lines were obtained between 400 and 600mμ with slopes of –0.0029 (in glucose), –0.0025 (in dextrine) and –0.0022 (in sucrose).

Other defined media were used with *C. mansoni*, including the *Neurospora* "minimal", and defined medium #2. In contrast to the results obtained with *Aureobasidium* the data in Table II show that differences between light- and dark-grown cultures can be observed on Defined medium #2 with *Cladosporium.* Abundant mycelium is formed by the organism on this medium and the cells are thin-walled and dark in color (Figure 2). Melanin is formed by cells in such cultures but no extracellular granules are found.

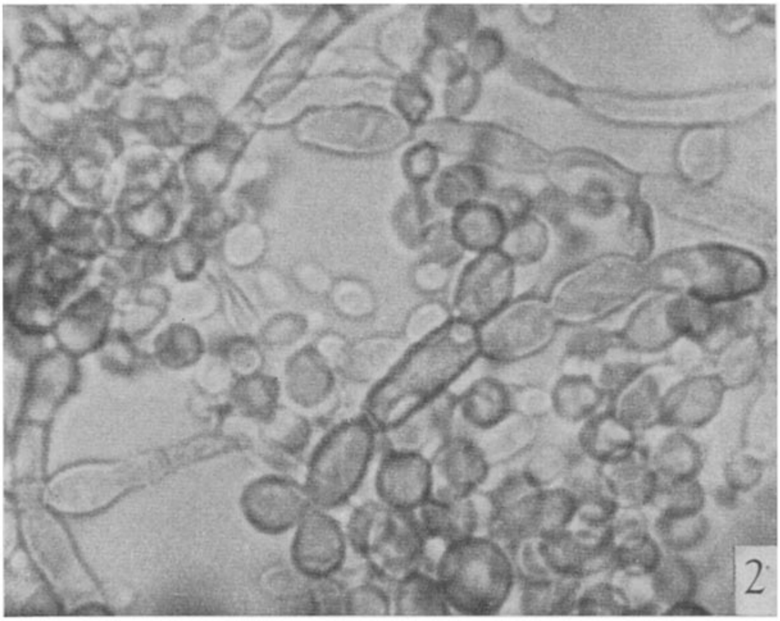

Fig. 2. Appearance of *Cladosporium mansoni* grown for two weeks in defined medium #2 in the light. Note the large amount of mycelium present under these conditions. Magnification — approx. 2,000 ×.

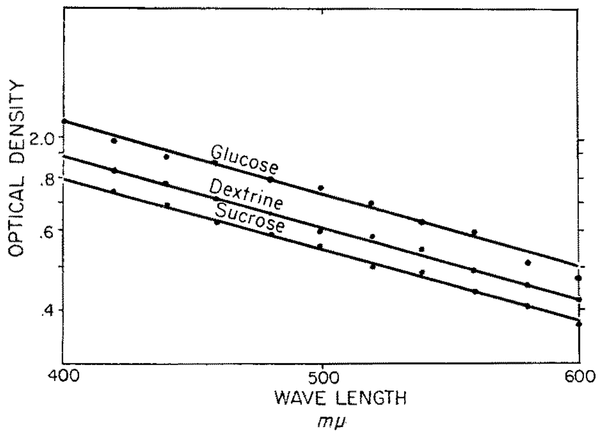

Fig. 3. Absorption spectra of alkaline extracts of cells of *Cladosporium mansoni* grown on basal medium #1 with 2 % dextrine. Cultures were maintained in the light for 14 days at 21°C and extracts were prepared and observed as described before in LINGAPPA *et al.*, 1963.

TABLE I.

Growth of C. mansoni on defined medium #1, containing 2% carbohydrate. Cultures were maintained in the light on a rotary shaker for 14 days.

Carbo-hydrate	Dry wt. of growth g	Wet wt. of Melanin g	Color of culture	Remarks
Dextrine	.1524	.0125	Greenish-black	Mycelium scanty, no extracellular granules, cells small, thin-walled, gray in aggregation.
Starch	.095	nil	no color	Starch granules incompletely used, cell yeast-like with a few brown cells, no mycelium.
Inulin	nil	nil	—	No growth
Chitin	nil	nil	—	No growth
Sucrose	.1139	.010	Light green	Cells yeast-like, small, colorless, or greenish; supernatant culture fluid light green.
Lactose	.0318	nil	Colorless	No mycelium, cells yeastlike, and colorless.
Maltose	.1371	.019	dark-brown	Cells yeast-like, thin-walled and light brown in aggregation; no mycelium; culture fluid brown.
Trehalose	.2126	.0325	brown	Cells were dark and supernatant; colorless) no mycelium.
Glucose	.1185	.0198	greenish	Scanty mycelium, yeast-like cells which are greenish in aggregation.
Fructose	.1391	nil	buff color	No mycelium; cells yeast-like; colorless.
Galactose	.112	.009	light brown	No mycelium, cells dark in aggregate; medium not colored.

Growth on defined medium #2 in the light was similar to that in *Neurospora* "minimal" medium in that considerable mycelium was found (Table II). Morphological similarities between the cells also exist in that they are of similar size and appearance, except that those in defined medium #2 are more uniform in both these respects.

Cultures grown in the dark on defined medium #2 revealed that light strongly influenced development in this medium. Such an effect is revealed in Table III where the morphology and size of cells of *Cladosporium* grown in the light and dark are compared. Whereas light-grown cells form abundant mycelium on defined medium #2, those grown in the dark are almost devoid of such aggregates. Moreover, "giant" cells, which sometimes reach over 20μ in diameter, are formed in the dark but are never found in the light. As can be seen in figure 1, partitions often appear in such "giant" cells,

TABLE II.

Effect of defined media upon growth and melanin formation by Cladosporium mansoni. Neurospora "minimal" medium and defined medium #2 were used and results are from cultures maintained in the light at 21°C, for 14 days.

Carbohydrate	Dry wt. of cells in g 45 ml culture	Wet wt. of melanin in 45 ml culture	Color of medium	Other remarks
Neurospora "minimal":				
Dextrine	.2445	.06	Color appeared on the 3rd day and became dark in one week.	Abundant mycelium; cells dark but thin-walled and 2—8μ in size. No granules.
Glucose	.1923	.065	Same as above	Same as above.
Defined medium #2:				
Dextrine & .2g/l NH_4NO_3 . . .	.3399	.0272	Dark green	Mycelium formed; no extracellular granules; cells uniform; 4μ in diameter.
2 g NH_4NO_3	.2929	—	Same as above	Same as above.
Glucose & .2 g/ml NH_4NO_3	.3025	.0205	Dark green	Same as above.

TABLE III.

Effect of light upon the size and types of cells formed by Cladosporium mansoni in defined medium #2 containing 2% dextrose and 0.2% NH_4NO_3. Cultures were maintained for 14 days at 21°C.

	Light-grown	Dark-grown
Type of Cells	Abundant mycelium, cells of which are uniform in size. (Fig. 2)	Very little mycelium; many "giant" cells. (Fig. 1)
Average size (100 cells)	$3 \times 5\mu$	$13 \times 14\mu$
Size range (100 cells)	$3 \times 7\mu$ — $5 \times 8\mu$	$8 \times 9\mu$ — $20 \times 22\mu$

suggesting the cleavage of protoplasm within a sporangium. However, the discharge of the contents of such cells was never observed so that their role is obscure.

Light was shown to influence the growth of *Cladosporium* on other media as well, for cultures grown on slants of nutrient, potato dextrose and corn meal agars, are very black after incubation in the light at the end of one week at 21°C. By contrast, those grown in the dark on these media require three weeks to turn black and differences in the morphology of cells also are observable.

TABLE IV.

Cladosporium mansoni in defined medium #1, containing 0.2 g NH_4NO_3 instead of asparagine. Cultures grown in the light for 14 days at 21°C,

Carbohydrate	Cell dry wt. in g (from 45 ml medium)	Melanin wet wt. in g	Culture color	Other remarks
Dextrine	.2433	.2576	Dark green	Cells uniform, yeast-like and extracellular granules present
Glucose	.1956	.2035	Dark green	Same as above except granular; extracellular granules were lacking.

Extracellular granules were not produced by *Cladosporium mansoni* in any of the media used above. However, it was found possible to induce their formation by using a modification of defined medium #1 in which 0.2 g per liter of NH_4NO_3 was used instead of asparagine. As the data in Table IV reveal, extracellular granules, of the size and color produced by *Aureobasidium*, are formed by *Cladosporium* in the above medium when dextrine is the carbon source. Light has no effect upon cell morphology or the formation of extracellular granules when defined medium #1, with NH_4NO_3, is used.

DISCUSSION

On the basis of their solubility, and the slope of the absorption spectra of basic solutions, the melanins from *Aureobasidium pullulans* and *Cladosporium mansoni* are similar. Thus, the slopes for the melanins from *A. pullulans* grown on sucrose, glucose and dextrine are —0.0018, —0.0017 and —0.0016 respectively (LINGAPPA *et al.*, 1963), as compared with —0.0022, —0.0029 and —0.0025 for the melanins from *C. mansoni* grown on the same media. On the other hand, these tests are non-specific and more analyses are necessary before a comparison of these melanins is possible in chemical terms.

Extracellular granules of the kind reported for *Aureobasidium* are formed by *Cladosporium* when dextrine is the carbon source in defined medium #1. However, such granules are formed only when NH_4NO_3 is the nitrogen source, in contrast to *Aureobasidium* which requires asparagine for their formation.

Among the differences between *Cladosporium* and *Aureobasidium* that are worth noting is that concerned with trehalose metabolism. Thus, dark brown cells form when *C. mansoni* is grown on trehalose, whereas uncolored yeast-like cells form in the other sugars in defined medium #1. By contrast, trehalose induces the formation of colorless cells of *Aureobasidium* whereas dark thick-walled cells form in the other good sources of carbon.

TABLE V.

Physiological differences between Aureobasidium pullulans and Cladosporium mansoni.

Physiological Property	Organism	
	*Aureobasidium pullulans**	*Cladosporium mansoni*
Effectiveness of carbohydrates in supporting growth:		
good sources	dextrine > starch > sucrose > maltose > glucose > trehalose > pectin fructose > galactose	trehalose > dextrine > fructose > maltose > glucose > sucrose > galactose
poor sources	inulin > lactose	starch > lactose, no growth in inulin.
Medium in which extracellular granules form	defined medium #1, dextrine with asparagine.	same, but with NH_4NO_3 as nit. source
Color of culture in light in defined medium #1 with trehalose ...	colorless	brown
Color of cells in above medium in light ...	colorless	brown
Light effects in:		
Neurospora "minimal medium" ...	+	—
nutrient agar ...	—	+
defined medium #1 ...	+	—
defined medium #2 ...	—	+

*Data taken from LINGAPPA *et al.*, 1963.

Light influences these two organisms differently as well in that the growth of *Aureobasidium* is affected in defined medium #1 and in *Neurospora* „minimal" medium. On the other hand, no light effects are discernible in *Cladosporium* on these media; instead, light effects are demonstrable on defined medium #2 and on nutrient agar. Conidial formation by *Cladosporium carpophilum* is reported to be stimulated by light (SCHWEIZER, 1958) but we know of no other effects of light upon members of this genus.

The data in Table V summarize the differences between *C. mansoni* and *A. pullulans* with respect to the experiments just discussed. Not only do these organisms differ in their ability to grow on certain carbohydrates, but the formation of pigment is affected differently by light and various media. We recognize that the great variability of these fungi, and their pleomorphism, restrict the generality of comparisons of only single strains. However, the physiological properties used in the present studies may lead to the development of reliable criteria for distinguishing between the black yeasts when these studies are extended.

Summary

Cladosporium mansoni (ATC #762) was shown to grow best on trehalose although a number of other carbohydrates are good carbon sources. On the other hand, starch, lactose and inulin support growth poorly, or not at all. In certain media light induces the formation of abundant mycelium whose cells are of average size; in the dark, little or no mycelium is formed and many "giant" cells appear. The pigment of *C. mansoni* was extracted and shown to have properties in common with those of melanins of other fungi. Extracellular granules, resembling melanin, appear in a defined medium containing dextrine and ammonium nitrate. A comparison between the response of *Cladosporium mansoni* and *Aureobasidium pullulans* to light and carbon sources shows several points of divergence.

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References

COOKE, W. B. (1962) An ecological life-history of *Aureobasidium pullulans*. Unpublished manuscript.

LINGAPPA, YAMUNA, SUSSMAN, A. S. & BERNSTEIN, I. (1963) Effect of light and media upon growth and melanin formation in *Aureobasidium pullulans* (De By.) Arn. (= *Pullularia pullulans*). Mycopathol. et Mycol. App. **20**, 109—128.

RYAN, F. T. (1950) Selected methods of *Neurospora* genetics. In *Methods in Medical Research*, Vol. 3. ed. Gerard, R. pp. 51—75. Chicago, U.S.A.: Year Book Publishers, Inc.

SCHWEIZER, H. (1958) Kulturansprüche des Kirschen- und des Pfirsichscherferregers [*Fusicladium cerasi* (RABH.) SACC. = *Venturia cerasi* A. D. und *Cladosporium carpophilum* J. THÜM] auf künstlichen Nährböden. Arch. Mikrobiol. **30**, 335—354.