GLASNIK BOTANIČKOG ZAVODA I BAŠTE UNIVERZITETA U BEOGRADU
BULLETIN DE L'INSTITUT ET DU JARDIN BOTANIQUES DE L'UNIVERSITÉ DE BEOGRAD
Tom I.I, 1—4, 1965—1966. (1968), Beograd

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# STUDIES ON THE EFFECT OF SOME SUBSTANCES AS POSSIBLE SUBSTITUTES OF LIGHT IN THE SPORULATION OF *ASPERGILLUS FLAVUS* 28-A

(Received October 4th. 1967)

To explain the complex effect of light on the behaviour of fungi several theories have been proposed. Carlile (1965) summarizes them as: »the hormonal theory«, suggested by Brauner (1954); »the inhibition theory«, formulated by Cochrane (1958); »the oxydation theory«, postulated by Coons (1916), and »the metabolite theory«, which supposes that light produces effects through inhibiting the formation, as well as through stimulating the production of substances which enable reproduction or other processes to occur. Besides these four theories it is worthy to mention another, pointed by Hawker (1957), which supposes that light must act by supplying energy leading to a photochemical reaction which is an essential step in the chain of metabolic processes involved in spore formation, and that with some fungi this energy source may be replaced to some extent by others, for example certain nutrients or increased temperature.

In a previous paper (Muntanjola-Cvetković 1968) we have stated that while studying 7 isolates of *A. flavus* the theory of Cochrane was confirmed, i. e. that light checks the vegetative growth and induces asexual sporulation.

In the present study we have tried to make an approach to the phenomenon of asexual sporulation in *A. flavus* 28-A, one of the most sensitive strains in our collection, in order to see whether the effect of light can be replaced by some other factors. These factors have been: two hormones — indol-3-acetic acid (IAA) and gibberellic acid ($GA_3$) —, and some nutrients — corn steep liquor (CSL) and a variety of sugars —, all of them added at various concentrations to a basal synthetic medium (Czapek's solution agar). Some additional experiments were made at different temperatures and different pH.

*IAA.* The action of IAA among fungi has been studied by Richards (1949), who established a stimulatory effect of this substance upon vegetative growth of *Phycomyces blakesleeanus,* and some inhibitory

responses on the germination and development of three other fungi (*Aspergillus candidus, Schyzophyllum commune,* and *Neurospora tetrasperma).* Machlis & Ossia (1953) found that IAA affects the development and maturing of meiosporangia of *Allomyces arbuscula.* Recently Kahn (1966) has reported that the addition of IAA in the synthetic nutrient solution induced sporulation in the dark and increased the growth rate of *Sclerotinia fructigena.* »Light and IAA appear to have the same effect on sporulation and one can be replaced by the other« — concludes Kahn.

$GA_3$. The results obtained by the authors who have investigated the effects of gibberellin or the *Gibberella fujikuroi* filtrates on different fungi provide several indications of:

1) its microbial inactivity (Brian et al. 1954, Borrow et al. 1955, Ciferri & Bertossi 1957);

2) its antagonistic action to several fungi (Edwards 1940, Koehler & Woodworth 1938, Slagg & Fellows 1947, Santoro & Casida 1962);

3) its stimulating effect on the sclerotium promotion in two fungi (Stowe & Yamaki 1957).

*Nutrients.* The view that spore formation depends on energy relationships raises the interesting question of whether this energy source can be replaced to some extent by others, for example certain nutrients.

As early as 1886, Brefeld stated that there was no point in taking into consideration external factors such as media, lack of oxygen and nitrogen, for the production of sexual or asexual organs in fungi, as only light had that influence. Lendner (1897), giving an interpretation of his own results, arrived at the conclusion that there were species of fungi obviously sensitive to light, but that sensitiveness would be greatly influenced by quality of substrata. Light — says Lendner — is only needed when media is unfavourable. In this case the fungus has to obtain, for its complete development, a certain amount of energy in the form of light. It would be natural — concludes this author — to associate this entire phenomena of light sensitiveness to a simple phenomenon of nutrition.

Coons (1916) considered that the effect of light may be replaceable by certain oxydizing agents. He stated that »the direct result of the presence of light is oxydation, and since a higher temperature or a richer food tends to bring about a higher oxydation and a more rapid metabolism, the increased reproduction would naturally be traced to the effect of oxydation«. Leonian (1924) observed that some fungal organisms which were not able to develop more than very few pycnidia in the dark and at the room temperature, or in the ordinary nutrient solution, gave rise to a greater abundance of fruit bodies when the temperature was raised, or when the concentration of the media was increased, even when light

was excluded. Houston & Oswald (1946) observed that the host tissue substitutes the light requirements of *Helminthosporium gramineum* for sporulation. This plant pathogen cannot sporulate on agar media in the absence of light, but it can do it in the dark if growing on the host tissue. Working on several Dematiaceae, Johnson & Halpin (1952) obtained evidence that the magnitude of conidial increase was markedly affected by substratum, but that the influence of depth of medium, humidity, pH, or quality of light, was negligible.

Raper, Fennell & Tresner (1953) reported the behaviour of *Aspergillus ornatus* strain 2256 to be strongly affected by light on the majority of media investigated: colonies produced very few or no conidial heads when incubated in the dark, whereas such structures were produced abundantly in parallel cultures incubated in diffuse daylight; nevertheless, Czapek's solution agar enriched with 1% corn steep liquor offered a striking exception: in the dark on this medium conidial production approached the level attained in light. »What is contained in steep liquor — say these authors — that enables the mold to circumvent, in large measure, the metabolic block which on other substrata effectively precludes the development of conidial structures in darkness?«

## MATERIAL AND METHODS

Organism: *Aspergillus flavus* Link, strain 28-A, isolated from local soils. As reported in previous papers (Muntanjola-Cvetković 1967, 1968) the responses to light and dark are the following opposite phenomena: *light* — mycelium, sclerotia, and pigments in the reverse of the colony very poor; conidial heads and conidia abundant and intensely green; *darkness* — mycelium, sclerotia, and pigment in the reverse of the colony abundant; conidial apparatus absent or scanty and pale colored; *alternating periods of light and darkness* — zonation. In the dark sporulation is suppressed, although the central region of the colony can be an exception, the sporulation being there more or less weak and never zonated when present. This phenomenon can be explained as a result of the mechanical injury to the mycelium when transferring the incipient colonies to the deffinitive plate. That sporulation may be stimulated in the border immediately surrounding a killed zone is a fairly well known phenomenon. The mechanical injury has been employed by several mycologists to provoke sporulation in some fungi whose fructifications are seldom and poorly obtained in laboratory (Billotte 1963).

Basal nutrient media:

(1) Czapek's solution agar with 3% sucrose;

(2) Czapek's solution agar with 20% sucrose.

Investigations have been made as comparative studies wherein the sucrose of the Czapek's agar was substituted by glucose, mannose or

raffinose as C source ,the pH not having been modified (pH 6.5), or having been depressed to 5.5 or increased to 8.5. This series of experiments was based on the studies by Hawker (1939, 1947) and Hawker & Chaudhuri (1946) on the nature of carbohydrates as a source of C, and their concentration in influencing reproduction in some Ascomycetes.

(3) Raulin's neutral solution agar;

(4) Basal nutrient media (1), (2), and (3), with the addition of water dissolved IAA in the following rates: 10, 20, and 50 p.p.m.;

(5) Basal nutrient media (1), (2), and (3) with the addition of $GA_3$ in the following rates: 10, 20, and 50 p.p.m.;

(6) Basal nutrient media (1), (2), and (3), with the addition of CSL obtained at different stages of the technological process of the corn wet--milling industry, all of them at two concentrations, 1% and 10%, and at three different pH: 5.5, 6.5, and 8.5*;

(7) Potato dextrose agar (PDA) (2% dextrose; pH 6.5);

(8) Malt agar (MA) (pH 6.5).

Amount of media: 30 cc per Petri dish.

Temperature of incubation: 25°C±1°C.

Incubation time: 8 days.

Illumination system: incandescent lamps mounted in a water-cooled glass tank, as described and figured in a previous paper (Muntanjola-Cvetković 1967).

Light conditions:

(a) 12 h white light 1.500 lx+12 h dark daily;

(b) continuous dark.

## RESULTS OBTAINED

1. No growth- or sporulation-promoting activity of IAA or $GA_3$ were detected at the rates of 10, 20 and 50 p.p.m. In the case of IAA these results contrast with those obtained by Kahn (1966) on *Sclerotinia fructigena.*

2. None of the sugars tested here can substitute the effect of light, but at high concentrations they may favour asexual sporulation in the dark, specially when the relative humidity of the atmosphere is low. Nevertheless, this sporulation is always much weaker than in light. If we establish a scale from 0 to 5 for sporulation intensity, this index can vary from 0 to 3 depending to the concentration of sugar and moisture conditions.

* The production and properties of CSL, and its usefulness in Microbiology, have been thoroughly discussed by Liggett & Koffler (1948).

Several samples from different periods of the steeping process, as well as the final concentrate, were employed for the present study.

The general analysis of this concentrate were the following ones: water 55%; proteines 21.37—23.34%; free reducing sugars 1.90—3.64%; lactic acid 8.44—8.88%; ash 10.26%; °Bx 50—52; pH 3.5—3.7. The concentrate was adjusted with destilled water until 12° Bé.

3. CSL conspicuously increases the diameter of the colonies when added to synthetic media; the rate of this increase has been dependent on the composition of media, and was more important in those with a lower concentration of sugars, as the following Table shows:

| | Diameter of the colonies (7 days old) in mm | | | |
|---|---|---|---|---|
| Media | Control | + 1%CSL | + 10%CSL | Relation |
| Raulin's neutral sol. agar | 35 | 58 | 60 | 1,60—1,70 |
| Czapek's sol. agar + 3% sugar | 50 | 75 | 75 | 1,50 |
| Czapek's sol. agar + 20% sugar | 65 | 75 | 75 | 1,15 |

4. The addition of CSL (at the rates of 1% and 10%) to the media with a low concentration of sugar (Raulin's neutral solution agar, and Czapek's solution agar + 3% sugar, both at different pH) was in no case able to substitute the effect of light. The addition of CSL to the media with a high concentration of sugar (20%) only enhances the picture obtained in the controls, i.e., there may be some sporulation in the dark, though it is always poorer than in light, not zonated, or it takes place over some sectors of the colony only. This effect may be favoured by dry conditions or by a high pH, and in that case conidial production in the dark may approach the level attained in the light. *A. flavus* 28-A shows some differences to that of *A. ornatus* reported by Raper et al.

5. This picture is a parallel one to that observed in other »natural« media — MA and PDA —, which are superior to synthetic ones as regards the diameter of the colonies, but they cannot completely replace the effect of light in promoting asexual sporulation in the dark.

6. Asexual sporulation in *A. flavus* 28-A takes place over a relatively wide range of pH. In our experiments the initial pH 6.5 of the nutrient media was deliberately varied from 5.5 to 8.5. The effect of a low pH is exerted principally upon the form of the colony, which appears more pleated and convex than the normal ones, and upon the colour of the conidia, which is more yellow than the normal green one; but sporulation is somehow affected too: at pH 5.5 it is slightly poorer than at pH 8.5.

7. When the temperature was raised to 30°C the sclerotia formed in the dark ripened quicker than in the colonies developed at 25°C, but this higher temperature had no effect on the increasing of sporulation.

## SUMMARY

*Aspergillus flavus* 28-A is a very light-sensitive strain which does not sporulate in the dark.

Neither indol-3-acetic acid nor gibberellic acid can substitute the effect of light and promote sporulation in the dark.

At high concentrations the sugars tested in this study (glucose, sucrose, raffinose, and mannose) may favour asexual sporulation in the dark.

The addition of CSL to the media was in no case able to substitute the effect of light; CSL only enhances the picture obtained in the controls. This picture is a parallel one with that observed in other »natural« media.

pH 8.5 has been more favourable for asexual sporulation than pH 5.5, and dry conditions more favourable than a wet atmosphere. Some other factors, such as mechanical injury, or the contact of another colony, stimulate asexual sporulation too.

By the combination of all these factors — high concentration of sugars, addition of CSL, high pH, dry conditions, mechanical injury — we can obtain colonies where asexual sporulation in the dark approaches the level of those incubated in the light.

When the temperature was raised to 30°C the sclerotia formed in the dark ripened quicker than in the colonies developed at 25°C but this higher temperature had no effect on the increasing of sporulation.

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## Rezime

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### PROUČAVANJE EFEKTA NEKIH SUBSTANCI KAO MOGUĆIH SUBSTITUENATA ZA SVETLOST U PROCESU SPORULACIJE *ASPERGILLUS FLAVUS* 28-A

*Aspergillus flavus* 28-A je linija koja je vrlo osetljiva na svetlost i koja ne proizvodi spore u mraku.

Ni indol-3-sirćetna ni giberelna kiselina ne mogu da zamene efekat svetlosti i da stimuliraju sporulaciju u mraku.

Šećeri koji su ovom radu ispitivani (dekstroza, manoza, saharoza i rafinoza) mogu u visokim koncentracijama da poboljšaju aseksualnu sporulaciju u mraku.

Dodavanje kukuruznog likera (»corn steep liquor«) u medijum ne može ni u kom slučaju da zameni efekat svetlosti; CSL samo pojačava sliku dobijenu u kontroli. Ova slika je paralelna onoj koja je zapažena sa drugim »prirodnim« medijumima.

pH 8.5 ima povoljnije dejstvo na aseksualnu sporulaciju nego pH 5.5 a suvi uslovi su povoljniji nego vlažna atmosfera. Neki drugi faktori, kao mehanička povreda, ili kontakt sa drugom kolonijom, takođe stimuliraju sporulaciju.

Kombinacijom svih ovih faktora — visokom koncentracijom šećera, dodavanjem CSL, visokom pH, suvim uslovima, mehaničkom povredom — mogu se dobiti kolonije kod kojih se nivo aseksualne sporulacije u mraku približava nivou kolonija koje se drže na svetlosti.

Kada se temperatura povisi na 30°C, sklerocije koje se formiraju u mraku sazrevaju brže nego kod kolonija držanih pri 25°C, ali viša temperatura ne pojačava sporulaciju.

(Iz Instituta za biološka istraživanja, Beograd)