Acta Biologica Szeged. 19 (1—4), pp. 79—91 (1973)

EFFECTS OF THE NUTRIENT COMPOSITION AND THE NATURE OF THE LIGHT ON THE GROWTH AND PIGMENT-SYNTHESIS OF SOME MOULDS

MÁRIA TÓTA and I. SZALAI

Department of Plant Physiology, Attila József University, Szeged

(Received September 25, 1972)

Summary

Investigations were carried out with the species *Penicillium notatum, Penicillium purpurogenum, Aspergillus repens* and *Aspergillus versicolor*. Their growth intensities and pigment-syntheses were studied as functions of the culture medium and the nature of the light. The findings were as follows:

1. The composition of the culture medium and the nature of the light each affect both the development and pigmentation of the fungi.

2. It can be said in general of the species included in the experiments that the growth of the colonies is completed in the 8th week.

3. Microelements added to the culture medium have different effects on the individual species, in some cases stimulatory, in others inhibitory. The growth is strongly inhibited by Zn, but slightly stimulated by Mn, and more strongly stimulated by Cu.

4. Comparison of the data show that *Penicillium notatum* is not light-sensitive, while the growth of *Penicillium purpurogenum* and *Aspergillus repens* is inhibited to a lesser extent by green and orange light, and to a greater extent by blue light; the growth is enhanced compared to that in white light by red light and darkness; an exception to this, however, is the growth in periodic illumination.

5. The light and culture medium also exert effects on the pigmentation.

6. The quantity and quality of the pigment content increase in every species during development.

7. The pigment composition of the *Aspergillus* species is more variable than that of the *Penicillium* species.

8. It is a generally valid tendency that the pigment content in orange and green illumination and in the dark is poorer than in white and red light.

9. The absorption curves of the pigments of colonies grown on various culture media show that the pigments synthetized by the fungi are mixtures of several components. No matter what the variations in the pigments, their absorption maxima lie in the range 400–550 nm. There is no absorption in the yellow, the red or the infrared.

Introduction

The important role of the pigments of various colours in the lives of plants is well known. Vital photosynthesis can occur only with the chlorophyll pigments. The presence of phytochromes is decisive in the development of many phenomena of life, such as photoperiodism, phototropism and the various photomorphoses. The carotinoids and flavins play a role as photoreceptors in the various movements. Finally, we can mention the biological importance of flavonoids, providing flowers with colour.

It is also well known that the various fungi are coloured by a whole series of pigments, but far less is understood about the varied forms of the pigments and their importance than for the higher-order plants, despite the large number of papers published on this topic.

Strains were selected for the experiments from the *Aspergillus* and *Penicillium* genera, partly on the basis of their rapid and simple cultivation, and partly because of the varied colours of their pigments. Accordingly, 10 *Penicillium* and 20 *Aspergillus* strains were selected, the most suitable of which proved to be the species

Penicillium purpurogenum	(787)*
Penicillium notatum	(190)
Aspergillus repens	(–)
Aspergillus versicolor	(710)

A decisive question with regard to the selection was the separability of the pigments. It was observed during the preliminary experiments that the pigments could not be successfully separated from their carriers with the organic solvents generally and widely used for pigment extracts. On the other hand, though it did prove possible to separate them by means of protein denaturing procedures (e.g. acidic hydrolyses), at the same time the pigments underwent changes in colour.

In accordance with our working hypothesis, a closer study was made of:

a. the effects exerted on the growth and the pigment-synthesis by culture media differing fundamentally from each other in composition;

b. the qualitative effects exerted by various monochromatic lights on the growth and pigment-synthesis in the case of strains cultivated on a given culture medium;

c. the intensity of pigment-synthesis and the quantitative relations in both cases, i.e. as functions of the culture medium and the light.

With the data obtained in our studies we wished to make a contribution to the elucidation of a region of plant physiology which has not yet been sufficiently investigated; it was assumed that the pigments are not useless metabolic products in the fungi, but metabolic products which are affected by the composition of the culture medium and the nature of the light, and which may play a role in the vegetative and reproductive development by means of their various absorption properties.

* The numbers in brackets denote the strain cultures maintained in the Viticultural and Oenological Research Institute.

The justification of our assumption is supported by a number of literature data. According to studies with microspores (CARLIE, 1965; MOHR, 1961), the light exerts significant growth-stimulating and growth-inhibiting effects alike. Since only the light absorbed by the cell is effective, it follows that the pigments may play a decisive role in both the growth and the multiplication of the fungi. Literature data can also be found which indicate that the light effect depends too on the composition of the culture medium (CARLILE, 1965; MUNTANJOLA et al., 1968). When cultivated on malt agar, *Penicillium clavigerum* is insensitive to light and grows with the same intensity in dark and light, whereas on CZAPEK's solution agar its rate of growth decreases on a 24-hour illumination, but remains constant on a daily 12-hour illumination. *Penicillium claviforme* requires light only at the beginning of development (CARLILE, 1965), until the mycelia have attained a length of some millimetres, and after this are apparently insensitive to illumination. In contrast, *Penicillium isariiforme* is sensitive to light throughout its entire life. What has been said would suggest that even the species belonging to a given genus react completely differently to light, depending at times on their developmental state, and at other times on the composition of the culture medium. On the other hand, this indicates that the sensitivity or indifference of the species to light is not connected with the kinship.

The synthesis of the pigments is explained by the „metabolism" theory in that the formation of certain metabolic products requires light and does not take place in the dark (CARLILE, 1965). It was observed on fungi grown in light and in dark that the development of their colours, and their varieties of colour, depended on the light.

It is a generally accepted view that light is necessary, primarily for carotenoid production. It has been found that *Fusarium aqueductum* (EBERHARD et al., 1961) and *Pyronema confluens* (CRALILE, 1956) synthetize carotenoid only in light, whereas light merely enhances the carotenoid synthesis in *Phycomyces blakesleeanus* (GARTON et al., 1951).

The light not only exerts an effect on the carotenoid-type pigments, but, for example, also inhibits the formation of naphthoquinone in *Fusarium osysporum*.

Melanin formation is affected by light in contrasting ways: it is stimulated in *Aureobasidium pullulans* (LINGAPPA, 1963), and inhibited in the black mutant of *Neuspora crassa* (RAPER, 1949). It is of great interest that pigments formed in the dark are reversibly transformed to other colours as a result of photo-oxidation if the fungus is illuminated. Several examples of this were found in our own experiments, in agreement with the data of RIEDHART and PORTER (1958), who observed the transformation of the yellow pigment in *Penicillium herguei* to a green pigment.

Also of interest are the results of a study of whether the photo-effect can be substituted, and if yes, to what extent with the change of the composition of the culture medium (MUNTANJOLA et al., 1968).

These observations show that none of the sugars is able to substitute the effect of light, but in high concentration a weak sporulation can be induced. In light, however, the composition of the culture medium causes changes in the pigmentation.

Besides the generalities, the literature does not provide sufficient information with regard to the effects of various monochromatic lights and various substrates on pigment-synthesis.

Materials and Methods

1. Experimental objects: *Penicillium purpurogenum, Penicillium notatum, Aspergillus repens* and *Aspergillus versicolor* strains. The strains were provided by the National Viticultural and Oenological Research Intstitute; for the duration of the experimental period they were kept on a potato glucose agar medium in test-tubes, under a protecting layer of paraffin oil, and these accurately determined strains were used for the transoculations.

2. Culture media: The following five culture media were used to study the effects of the medium:

a. Czapek-Dox solid culture medium (pH 6.8—6.9) to which the sugar was added before the sugar was added before the final sterilization.

b. Czapek-Dox culture medium supplemented with microelements in the following variations:

1.	Basal nutrient medium (a) + $CuSO_4 \cdot 5\,H_2O$	0.0025	g
2.	Basal nutrient medium (a) + $MnSO_4$	0.0025	g
3.	Basal nutrient medium (a) + $ZnSO_4$	0.0025	g
4.	Basal nutrient medium (a) + $MnSO_4$	0.001	g
	+ $ZnSO_4$	0.001	g
	+ $CuSO_4 \cdot 5\,H_2O$	0.001	g

c. Potato agar culture medium (Szalai and Frenyó, 1962).

d. Czapek-Dox liquid culture medium.

e. Modified Czapek-Dox culture medium, the basal composition of which agreed with that of culture medium (a), but the C cource was varied and it was supplemented with vitamin B in the following variations:

(1) Varying the C source, 10% or 20% dextrose was employed in place of 30 g (3%) dextrose.

(2) Culture medium (c) was supplemented with a yeast extract.

The culture media were freshly prepared in all cases and, after adjustment to the appropriate pH, were poured according to the aims of the experiment into test-tubes 1.5 cm in diameter, Petri dishes 10 cm in diameter, or 150 ml Erlenmeyer flasks.

After sterilization the culture media were put into room-temperature thermostats for 3—4 days, and only those were used for inoculation which proved to be sterile. The spore or mycelium pieces were transferred onto the appropriate sterile culture medium in a UV-sterilized chamber by means of an inoculating loop, in such a way that it adhered well to the surface of the agar, but did not sink in deeply and into the culture medium.

Under the (usual) sterile conditions, infection-free cultures were atteined in 80% of the transoculations.

The solid culture media were used to study the intensity of growth and the structure of colony formation, while pigments were extracted from the cultures of the liquid media.

In both cases 30 ml of nutrient solution was employed.

3. Cultural procedure (incubation)

The cultural conditions were varied according to the aims of the experiment. The cultures were placed in thermostats, kept in the dark, and developed at 26—28 °C (± 0.2 °C). The cultures subjected to variation by night and day were maintained at laboratory temperature of 22—33 °C.

For the study of the photo-effect the cultures were placed in a climatic chamber, the temperature of which was similarly 26—28 °C (± 1 °C). The climatic chamber was illuminated with light of an intensity of 1200—1500 lux, enriched with monochromatic light by the use of white, red, orange, green and blue discharge tubes.

The growth of the cultures was expressed with the index of transverse diameters, and the measurements were made at gradually increasing intervals (on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th days).

4. Pigment extraction

The cultures on the liquid culture medium were separated on a Büchner funnel on the intense pigmentation of the culture medium (generally after 14—16 days), the mycelium mass homogenized, and the pigments were dissolved out by treatment with methanol. Two methods were used during the procedure. In one case the culture was subjected to a fractionation procedure, an aqueous extract (fraction II) was prepared from the mycelium mass after homogenization, and the residual pigments were eluted with methanol during 24 hours (fraction III). The nutrient solution separated from the mycelium mass (fraction I) was subjected to spectrophotometric analysis after evaporation, similarly to the other two fractions.

In the other case the methanolic extract of the mycelium was combined with the filtrate, and used for chromatography after methanolic extraction and mild evaporation.

5. Chromatography of the pigments

Thin layers were prepared from silica gel according to the method of STAHL (1967) and applied in a thickness of 1 mm to 20×20 cm glass plates. After drying for 10 minutes the plates were activated at 50 °C. 0.1 ml portions of the material were transferred to the start-point by micropipette and a chloroform : acetic acid mixture (7 : 2 v/v) was used as solvent; this had been found to be the most suitable after trials with a large number of running solvents. The development was carried out at room temperature, a time of 80—90 minutes being required for the attainment of a front distance of 15 cm.

The fractionated pigment components were also studied in UV light.

Results and discussion

Dependence of growth on the composition of the culture medium

For every culture medium it can be said that the colonies of the species selected for the experiment grew quickly and the pigmentation, zonation and colony character typical of the species had developed on the 6th day following the transoculation. After the 6th day the inner parts of the colony multiply in thickness, but in contrast the peripheral part remains thinner and the hyphal mat is looser. The colony becomes increasingly thick on ageing.

It is also generally valid for the cases studied that the growth of the

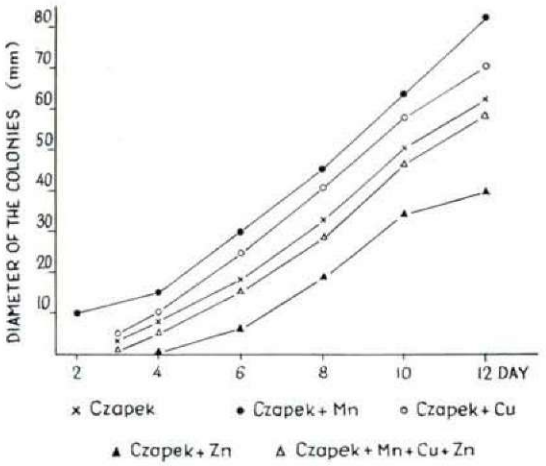

Fig. 1. Growth of *Penicillium notatum* in white light on Czapek-Dox agar culture medium enriched with microelements.

colony is completed in the 8th–10th week, the mycelia harden, and their water content decreases.

The growths of *Penicillium notatum* and *Pencillium purpurogenum* on Czapek-Dox agar were measured in white light and the obtained results were used as controls for the growths on culture media of different compositions (Figs. 1, 2).

Of the microelements, Zn inhibited the growth, while Mn stimulated it to a greater extent and Cu to a lesser extent compared to the basal media. The use of the three microelements together did not result in as large a growth as for Mn or Cu alone; thus, the inhibiting effect of Zn was exhibited even in the combination of the microelements (Fig. 1).

The growth of *Penicillium purpurogenum* was similar to that of *Penicillium notatum* on the basal medium. The inhibiting effect of Zn is striking, particularly at the beginning of the development, and in this case the stimulating effect of Cu exceeds that of Mn. It is worthy of note that the combined application of the three microelements stimulates the growth in the first ten days of the development better than the microelements separately, and the inhibiting effect of Zn appears only in the later stage of the development (Fig. 2).

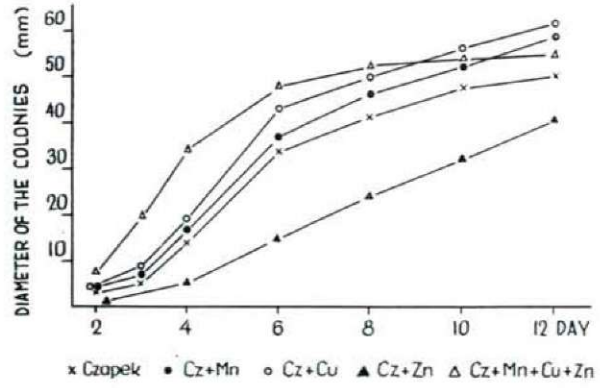

Fig. 2. Growth of *Penicillium purpurogenum* in white light on Czapek-Dox agar culture medium enriched with microelements.

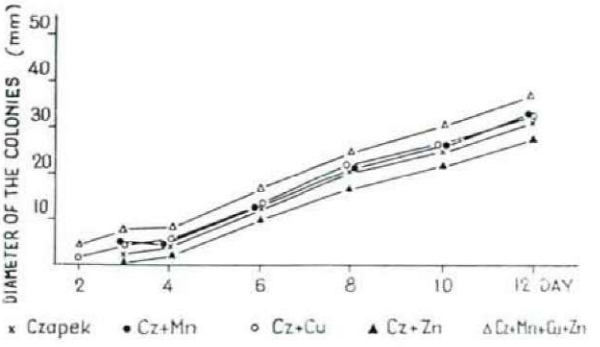

Fig. 3. Growth of *Aspergillus repens* in white light on Czapek-Dox agar culture medium enriched with microelements.

The composition of the culture medium has only an extremely small effect on the growth of *Aspergillus repens*. The curves shown in Figure 3 are practically parallel to each other, and the differences between them are so small that they may be considered to be within the limits of error. With the exception of Zn, the microelements exert a just measurable stimulation, while Zn exhibits an extremely slight inhibiting effect.

As regards the given culture media, therefore, *Aspergillus repens* is not sensitive.

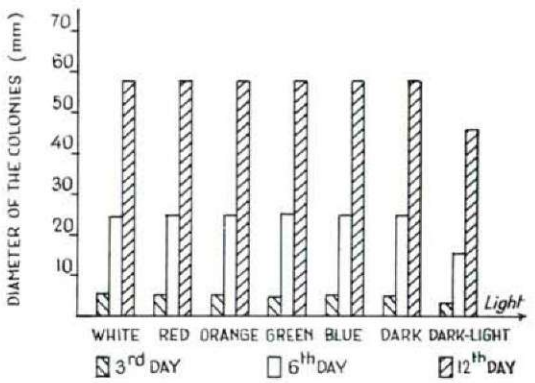

Fig. 4. Growth of *Penicillium notatum* on Czapek-Dox agar culture medium in various monochromatic lights.

Photo-effects on the growth on Czapek-Dox agar culture medium

Significant differences in growth were observed in the various spectral regions enriched with monochromatic light as detailed in the methodological description (Figs. 4–6). For the better illustration of the differences column graphs were used, and to show the differences more distinctly only the experimental data for the 3rd, 6th and 12th days are given. Growth in white light was again taken as control, and the growths observed in the monochromatic light was compared to this.

In the case of *Penicillium notatum* the monochromatic light induced no stimulation or inhibition at all, but in periodic illumination the growth decreased, and thus *Penicillium notatum* can be considered a photophilic fungus. This photophilia, however, can be satisfied not only by white light but by any monochromatic light. The 1-mm differences occurring here and there in the colony diameter are probably due to errors of measurement (Fig. 4).

The photo-effects are just the reverse in the case of *Penicillium purpurogenum*. If the growth observed in white light is used as control, then the growth in the dark can be said to be more intense than in light. The strong decrease of the growth measured in periodic illumination can not be explained by the scotophilia. Of the monochromatic light regions, red light has almost the same effect as white light, while orange, green and blue lights exhibit

increasing inhibiting effects on the growth. It can be concluded from the experimental data that the growth of *Penicillium purpurogenum* is inhibited to an increasing extent with the shortening of the wavelength of the light (Fig. 5).

Aspergillus repens behaves similarly to *Penicillium purpurogenum* and grows much more quickly in the dark than in constant white light. The growth is also greater in red light than in white light, but decreases in all the other spectral regions in proportion to the shortening of the wavelength. In periodic

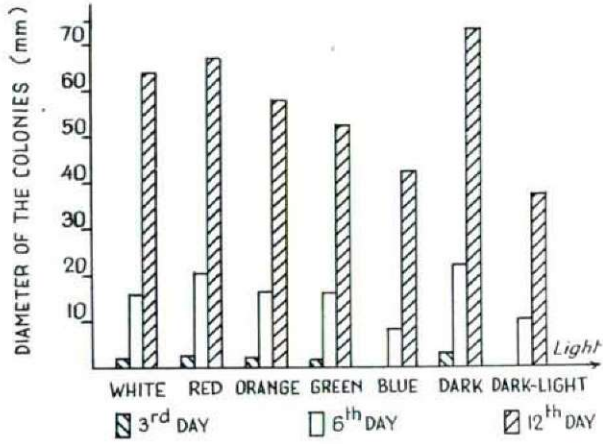

Fig. 5. Growth of *Penicillium purpurogenum* on Czapek-Dox agar culture medium in various monochromatic lights.

illumination the growth-stimulating effect of the dark period is more weakly manifested than the inhibiting effect of the light (Fig. 6).

It can be established from the above that for the three moulds examined

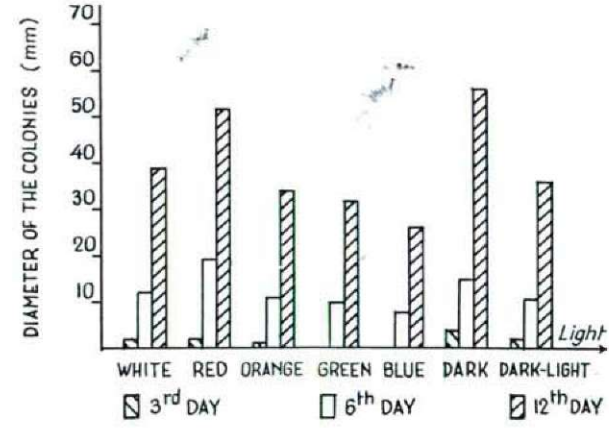

Fig. 6. Growth of *Aspergillus repens* on Czapek-Dox agar culture medium in various monochromatic lights.

the nature of the light plays a larger role in the regulation of the rate of growth than the composition of the culture media studied.

The next question is whether this effect of the two factors occurs in the synthesis of the pigments.

Photo-effects and variation of the pigment content

Unexpected difficulties were encountered in the extraction and separation of the pigments, and this compelled us to simplify the planned complex investigations. The difficulties arose from the fact that the chromatographic separation of the components succeeded only after long experimentation, as already indicated in the methodological part. Even with extraction and running in decreased light, the individual colour components, although always identifiable, appeared with very faint colours, and in ordinary light the contours of the spots could not be identified in most cases. It was effective in almost every case to study the thin layers in UV light (254 m), when the colours were intensified and the contours too became distinguishable. The following can be said in connection with the pigment variation:

In *Penicillium notatum* a maximum of 7 spots could be distinguished in the red on the 14th day. Pigments of colonies grown in orange illumination gave 5 spots. The pigments synthetized in the different illuminations are given in Figure 7, and we should like to indicate the differentiation og these. The chromatogram confirms that the colour composition of *Penicillium notatum* varies in quality primarily depending on the illumination.

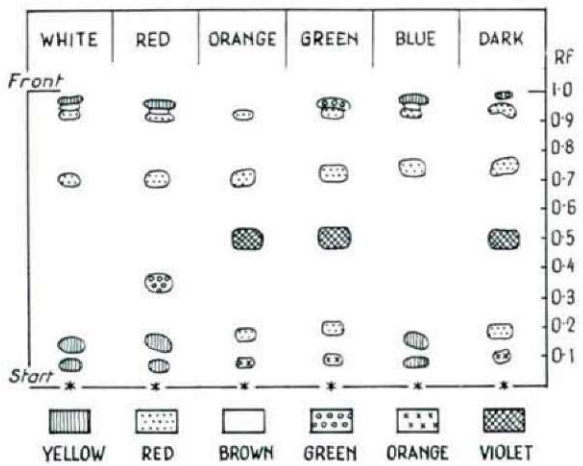

Fig. 7. 14-day *Penicillium notatum* pigments separated on a silica gel thin layer.

The number of components increases in the pigments extracted on the 24th day; this is most striking in colonies developing in the dark, where 10 components can be recognized, and becomes predominant in red, brown and bluish-violet colours. It can be stated in connection with *Penicillium notatum*

that in the older colonies the green and *bluish*-violet components accumulate (Fig. 8), these not being characteristic for young colonies (Fig. 7).

The richness of colour of *Penicillium purpurogenum* is greater than that of *Penicillium notatum.* A maximum of 9 and a minimum of 6 colour-spots were distinguished on the 14th day. The most spots occurred in blue and white lights. The fewest spots were given by the chromatogram of an extract of fungus grown in the dark. In contrast with *Penicillium notatum,* the green spot here with an Rf value of 0.37 appears for all of the monochromatic lights.

In *Penicillium purpurogenum* the red colour exhibits three different Rf values, and is present in such large quantity that the culture medium is coloured a completely homogeneous red. In the dark, or in orange and green lights a green pigment appears at Rf 0.12; this is not present in red light.

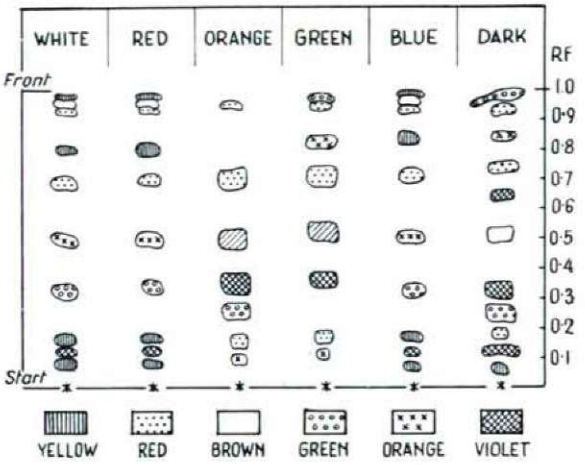

Fig. 8. 24-day *Penicillium notatum* pigments separated on a silica gel thin layer.

The chromatogram of pigments extracted on the 24th day is even richer in colour, and the number of components is higher. At most 12 components appear; this in due in part to new red spots and shades of green. In the dark, however, the pigment content remained unchanged even on the 24th day.

In *Aspergillus repens* a minimum of 4 and a maximum of 8 components appear on the 14th day: the fewest in orange or darkness, the most in red light.

On the 24th day 13 pigments are found in the cultures in blue light, and only 8 pigments in those in the dark. The compositions of the pigments exhibit many similarities in white, red and blue lights, and in orange and green lights.

The pigment content of *Aspergillus versicolor* is the most varied, 14 pigments appearing in the 14-day culture.

The most varied pigment content is synthetized in white light, and the least (5 spots) in the dark. In this respect, therefore, the two *Aspergillus* are similar to each other. The variety of the pigments increases with the growth

of the culture; the red, and then the yellow and yellowish-green pigments dominate, while the blue pigments have the lowest contents (Fig. 9). The young colonies are richer in colour in red light than in blue light, but the situation is the reverse in the older colonies (Fig. 10).

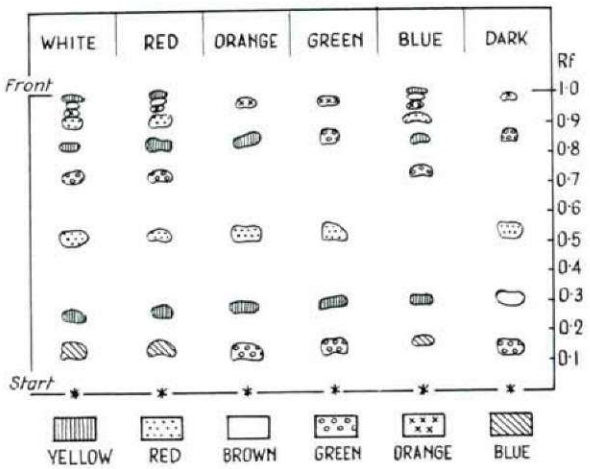

Fig. 9. 14-day *Aspergillus versicolor* pigments separated on a silica gel thin layer.

There appears to be a generally valid tendency for the pigment content to be poorer in orange and green lights and in the dark, whereas it is the most varied in white or red lights. The pigment compositions of the *Aspergillus* species are more varied than those of the *Penicillium* species.

It is characteristic for the individual species that they give a definite colour to the culture medium in their environment, this pigment being synthetized and excreted by the mycelium before or simultaneously with the spo-

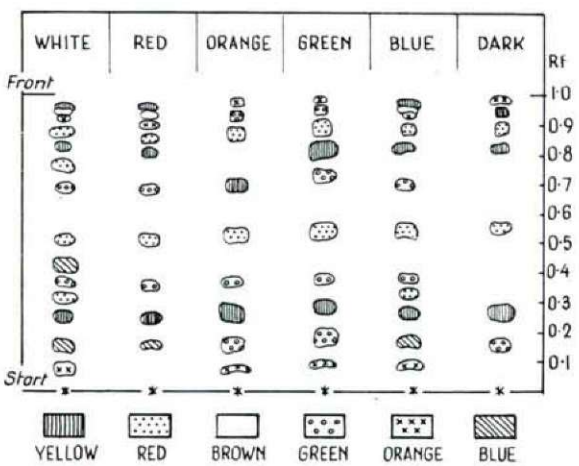

Fig. 10. 24-day *Aspergillus versicolor* pigments separated on a silica gel thin layer.

rulation. The pigment-synthesis and excretion by the mycelia is continuous, for the pigmentation of the nutrient solution becomes more intense.

The complexity of the absorption spectra indicate that the apparently homogeneous pigments produced by the mycelia are mixtures of several components. For technical reasons we have not yet been able to make a separate study of the pigments of the conidia, and so it cannot be estableshed from the chromatograms which parts of the fungus the individual pigments are derived from. The aim of our further studies is to separate the pigments if possible according to organs and to identify the individual organs chemically.

Effect of the composition of the culture medium on the pigment-synthesis

Change of the composition of the culture medium also leads to changes in the composition of the pigments.

Absorption spectra were taken in accordance with the fractions described in the methological part. The extenctions of fraction I of the pigments excreted into culture media of various compositions are shown in Figure 11. It can be seen that the excreted pigments are composed of several colour-components, the absorption maxima of which lie in the wavelength region 400–500 nm. The nature of the curve is the same in the case of the different culture media.

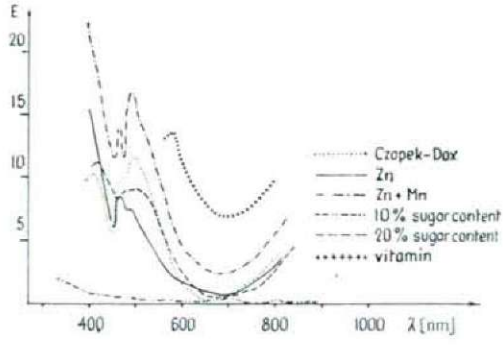

Fig. 11. Extinctions of *Penicillium purpurogenum* pigment extract after growth in nutrient solution of various compositions.

It appears that if a microelement such as Zn is added to the culture medium the value of the extinction decreases, i.e. the amount of pigment diffusing out into the nutrient solution decreases. The joint application of Zn and Mn promotes the excretion. As we have seen, the synergistic effect of the two microelements is also manifested in the growth of the mycelium.

With the increase of the sugar content of the culture medium the character of the curve does not alter, but in contrast the diffusing out of the pigment is inhibited. In sugar-rich culture media the bulk of the pigments is found in fractions II and III.

It is assumed that there is a relation between the increase of the sugar concentration and the retention of the pigment, presumably as a result of the change of the permeability or the stronger bonding of the pigments. However, the relation is not known.

References

CARLILE, M. J. (1965): The photobiology of Fungi. — Annual Review of Plant Physiology *16*, 175—199.

CARLILE, M. J. and J. FRIEND (1956): Carotenoids and reproduction in *Pyronema confluens*. — Nature *178*, 369—370.

EBERHARD, D., W. RAU und C. ZEHENDER (1961): Über den Einfluss des Lichts auf die Carotinoidbildung von *Fusarium aqueductum*. — Planta *56*, 302—308.

FFIEDERICKSON, I. and H. ENGEL (1960:) Der Abschussrhytmus der Fruchtkörper von *Sphaerobulus stellatus* (THODE) PERS. — Planta *55*, 313—326.

GARTON, G. A., T. W. GOODWIN and W. LIJINSKY (1951): Studies in carotenogenesis. 1. General conditions governing β-carotene synthesis by the Fungus *Phycomyces blakesleeanus*

GARTON, G. A., T. W. GOODWIN and W. LIJINSKY (1951): Studies in carotenogenesis. NTAJA BURGEFF. — Biochem. J. *48*, 154—163.

JOHNSON, T. W. and J. E. HALPIN (1952): Influence of light on the morphology and production of conidia in some species of *Dermatiaceae*. — Phytopath. *42*, 342.

MOHR, H. (1961): Wirkungen kurzwelligen Lichtes. In Handbuch der Pflanzenphysiologie, XVI. 439—531 (RUHLAND, W. Ed., SPRINGER, Berlin).

MUNTANJOLA, M., M. CVETKOVIČ and M. NESKOVIČ (1968): Studies on the effect of some substances as possible substitutes of light in the sporulation of *Aspergillus flavus* 28—A. — Bull. Inst. et Jard. Bot. Univ. Belgrade 2 n. s. 35—41.

MUNTANJOLA, M., M. CVETKOVIČ, M. NESKOVIČ and L. J. CULAFIC (1968): Morphogenetic responses of *Aspergillus aureolatus*. — Bull. Ints. Jard. Bot. Univ. Belgrade, 3 n. s. 29—34.

NARASIMHARACI, N. and L. C. NINING (1963): Studies on the pigments of *Penicillium herquei*. — Canadian J. Chem. *41*, 641—648.

RAPER, K. B. and C. THOM (1949): A manual of the *Penicillia* Baltimore.

RAPER, K. B. and C. THOM (1949): A manual of the *Aspergilla* Baltimore.

STAHL, E. (1967): Dünnschicht-Chromatographie. — Springer Verlag, Berlin.

SZALAI, I. és FRENYÓ, V. (1962): Növénytani praktikum II. Tankönyvkiadó, Budapest.

VÖRÖS, J. és G. UBRISZY (1960): A penészgombák. I. 8. Budapest.

Addresses of the authors:

MÁRIA TÓTA

Prof. Dr. I. SZALAI

Department of Plant Physiology
A. J. University,
H — 6701 Szeged P. O. Box 428,
Hungary