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

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MORPHOGENETIC RESPONSES OF *ASPERGILLUS AUREOLATUS* MUNT.-CVET. & BATA TO DIFFERENT CARBOHYDRATES AND LIGHT

(Received March 1st. 1968)

While studying the morphological characteristics of *Aspergillus aureolatus* on the standard media given by Thom & Raper (1945) and Raper & Fennell (1965) for identification of Aspergilli, a considerably different growth pattern was remarked between colonies cultivated on Czapek's solution agar with 3% sucrose (where they are plane, slow growing, with scanty mycelial production and sporulation) (Fig. 1) and those cultivated on malt extract agar or PDA (where growth is abundant, pigment formation of the immersed hyphae intense, and central areas of the colonies show a tendency to split) (Figs. 2, 3, 4).

It was also remarked that sporulation in *A. aureolatus* is not a light-dependent phenomenon, because it occurs in the dark as well as in the light, and zonation, which in *A. aureolatus* takes the form of a fairly regular rings of crowded and sparse conidial structures, was not a response to external factors.

The present paper reports the results of further investigations on these subjects.

MATERIAL AND METHODS

Organism: *A. aureolatus*, isolated in Belgrade in 1963.

Culture media: Czapek's solution agar+sucrose, or the sucrose of the routine formula has been replaced by other sugars. The sugars and their concentrations employed in the present study are the following: dextrose, fructose, mannose, sucrose, lactose, and maltose, all of them at 3%, 10%, and 20%. In each case two parallel series were maintained under the same conditions of temperature (25±1°C): one of them was kept in continuous darkness, while the other received 12 hours of white light (1.500 lx) daily.

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

Determination of sugars: Agar surrounding the colonies was extracted with water or methanol, solvent evaporated at reduced pressure, and the residue spotted on 250 μ thick silica-gel G layers, buffered with 0,02 M sodium acetate. A mixture of chloroform : methanol (60 : 40) was used as solvent. Sugars were detected after spraying the plates with anilin-diphenylamine-phosphoric acid reagent (Stahl 1967). In some experiments, instead of spraying, silica-gel was scraped off and eluted with 1 ml of water, and the amount of reducing sugars was determined with Somogyi—Nelson reagent. Optical density was read at 520 mμ.

RESULTS

THE EFFECTS OF CARBOHYDRATES

Hexoses

Colonies grown on Czapek's solution agar with dextrose, fructose, or mannose follow the same pattern, which is enterily different from the one given by sucrose or lactose.

Though slow growing, colonies on Czapek's solution agar with *dextrose* are luxuriant, densely sporulated and surrounded by a gold-yellow halo of immersed hyphae. The most striking character of the colony surface is the complete and dramatic split of the central area, which leaves a large hollow (Fig. 5); the following zone is convex and covered by asexual fructifications; the immersed mycelium extends beyond the area of surface growth, often in a lobed or arborescent pattern (Fig. 2). In the dark, sporulation is so dense that zonations are indistinguishable. Colonies submitted to the light-cycle differ from those cultivated in darkness because: 1) growth is more moderate; 2) the central hollow is not so big and appears later (Fig. 6); 3) zonations are fairly evident. The diameter of colonies and the importance of the central hollow depend on the concentration of dextrose added to the mineral solution.

Colonies grown on the same media with *fructose* instead of dextrose show limited differences from the above description. They are less raised at center, especially when cultivated in light; the orange pigment of the immersed hyphae is more dull.

On *mannose* too, the central part of the colony splits earlier in cultures grown in the dark than in those receiving daily light-treatment (Figs. 7 and 8). Pigmentation of the immersed hyphae is more intense and brilliantly reddish-orange on mannose than on the other hexoses.

Disaccharides (except maltose)*

Growth on Czapek's solution agar with 3% *sucrose* is very thin, with vegetative mycelium largely immersed and bearing conidial structures of rather small dimensions. Colonies develop slowly and present the general aspect of a mold suffering from some nutritional defficiency. The yellow pigment of the immersed hyphae is seldom formed; still, in old colonies this pigment is eventually to be observed in the marginal areas.

Colonies on Czapek's solution agar with 3% *lactose* bear a resemblance to those on Czapek's sucrose agar with regard to growth habit. But here the production of mycelium is still poorer, never pigmented, and conidial heads are significantly small. A higher concentration of lactose in the medium does not increase growth or sporulation, as in the case of sucrose occurs. Zonation is evident (Figs. 9 and 10); zones begin to be formed 48 h after transfering the colony to the definitive plate, and their periodicity can be followed daily. In a colony which has reached its maturity we can distinguish: 1) a central area where sporulation is more dense and zonations almost undistinguishable because their coalescence; 2) a following area showing evident zonations; 3) an outer area where the loss of growth activity and beginning of senescence is evident.

These results reveal a poor capacity of *A. aureolatus* to utilise disaccharides as sole source of C, especially in the case of lactose. As for sucrose, this ability was somewhat stronger in 1963, when *A. aureolatus* was firstly isolated, than now ,after five years on artificial culture.

It has been observed that the presence of some other fungi in the same plate where *A. aureolatus* was cultivated on Czapek's solution agar with 3% sucrose profoundly influences the behaviour of the latter. Among these fungi we have identified *Penicillium implicatum, P. notatum, Cladosporium* sp., and some others. The effects of these fungi are reflected on the production of a richer vegetative growth and sporulation, as well as on the formation of pigment in the immersed mycelium (Figs. 11, 12). In considering the strong responses of *A. aureolatus* to hexoses, it was assumed that enzymatic activity of the above mentioned Penicillia and *Cladosporium* could hydrolise the sucrose of the media and supply *A. aureolatus* with a certain amount of monosaccharides, or some products of the glycolytic processes, necessary for conidial apparatus and pigment formation. Some tests were performed to prove the correctness of this interpretation. Single colonies of the above mentioned Penicillia or *Cladosporium* were cultivated on Czapek's solution agar with 3% sucrose; after 15 days the agar surrounding the colonies was extracted with water or methanol, and the extract was completely evaporated at reduced pressure. Bidestilled water was added to the residue, and the aqueous solution

* Colonies on Czapek's agar with 3% maltose instead of sucrose were slow growing and very restricted, with convex surface and irregular borders surrounded by an incomplete orange halo of immersed hyphae, central areas split or not, but their general aspect recalling those grown on hexoses. Chromatographic tests of pure solutions of the maltose employed (Carlo Erba) showed the presence of not negligible quantities of dextrose as impurity. For this reason, experiments made with maltose as sole source of C are not taken here into account.

was then sterilized. The effect of this solution was investigated by the cylinder plate method: the solution to be tested was placed in the hollows made in the Czapek's solution agar + 3% sucrose contained in a Petri dish; 3 cm far from the hollow was inoculated a 48 h old colony of *A. aureolatus*. Diffusing into the agar, the extract solution revealed its potency enhancing sporulation as well as pigment formation in the immersed hyphae of the half section of the *A. aureolatus* colony facing to the hollow (Fig. 13). This test proved two properties of the active substance: 1) its solubility in water and methanol; 2) its thermostability. Chromatographic tests revealed the presence of sucrose, fructose, and dextrose on the extract solution tested. The more the agar tested was close to the colony of Penicillia or *Cladosporium* the less was the presence of sucrose important. Two controls were investigated: № 1, extract of the Czapek's solution agar + 3% sucrose surrounding the colonies of *A. aureolatus;* and № 2, extract of the Czapek's solution agar + 3% sucrose after sterilization. Table 1 shows the amounts of reducing sugars found in those extracts. In each case agar from four colonies was extracted, extract residues dissolved in 3 ml of water, and 0,1 ml of these solutions spotted on a plate.

Table 1

Amount of reducing sugars expressed in mg dextrose equivalents per one culture

Colonies of	mg dextrose equivalents
P. implicatum	50,25
Cladosporium sp.	38,33
A. aureolatus (15 days old colonies)	4,52
Control (Czapek's sol. agar + 3% sucrose)	5,52

Somogyi—Nelson reaction revealed the presence of certain amount of hexoses in 25 days old colonies of *A. aureolatus* which exceptionally produced the yellow halo of immersed hyphae on Czapek's solution agar + 3% sucrose.

Some additional tests were performed wherein the hollows made in the Czapek's solution agar + 3% sucrose as described above were filled with pure solutions (3%) of dextrose, fructose, sucrose, or lactose. The solutions of dextrose and fructose proved to be highly effective, as the half section of the *A. aureolatus* colonies facing to the hollows presented a dense sporulation and intensely orange immersed hyphae after 3 days, though in the other half of the colonies growth was scanty and the immersed hyphae not pigmented. Pure solutions of sucrose or lactose did not produce these effects.

It is unquestionable that some other substances are segregated by the Penicillia or the *Cladosporium* to which we refere in the present work,

and that these substances may be active in sporulation or pigment promotion, but in this study we have focused our attention to the effect of carbohydrates and proved their significance for sporulation and pigment formation in *A. aureolatus*.

THE EFFECT OF LIGHT

While sporulation of some fungi is greatly dependent on light, with others the development of fruit-body initials may be inhibited by light, at least to some extent. *A. aureolatus* is an example of these last ones: colonies grown in the dark on a favourable medium are densely sporulated, while those in light are somewhat poorer in conidial apparatus production; the intensity of this effect is correlated to the composition of the culture media.

In the case of the species with a light-dependent asexual sporulation, which can be examplified by *A. flavus* strain 28-A (Muntanjola-Cvetković 1968, Muntanjola-Cvetković & Nešković 1968), alternating periods of light and darkness give as a result the zonation of the colonies. With *A. aureolatus* zonation is evident even in continuous dark, and this fact denotes an endogenous cycle, in which the rythm has a value equal to the diurnal periodicity (Figs. 9, 14, 15).

The evidence of zonation in *A. aureolatus* very much depends on the culture media. The nature firstly, and the concentration secondly, of carbohydrates as sole source of C greatly affect the development and aspect of colonies. Since growth of *A. aureolatus* is poorer on Czapek's solution agar with 3% sucrose or lactose than on the same substrate containing instead dextrose, fructose or mannose, the expression of zonations is more conspicuous in the first case because coalescence of the rings is avoided. Still, in old colonies on richer media, as for instance on PDA, zonations are to be seen in the marginal area (Fig. 15).

SUMMARY

1. The ability of *A. aureolatus* to use carbohydrates as sole source of C varies according to the nature of the sugar: hexoses are readily utilised, but not disaccharides (especially lactose). Colonies grown on Czapek's solution agar with some hexose differ considerably from those developed on the same medium when sucrose or lactose are the source of C.

2. Some organisms (among them *Penicillium implicatum*, *P. notatum*, *Cladosporium* sp.) capable to hydrolise the sucrose of the Czapek's solution agar enhance conidial structures formation and the production of the yellow-orange pigment of the immersed hyphae of *A. aureolatus* when growing in the same plate. Extracts of the secreted products of these other organisms are active too to promote sporulation and pigment formation in colonies of *A. aureolatus* growing on Czapek's solution agar with 3% sucrose or lactose. Chromatographic and chemical analysis of these ex-

tracts have revealed the presence of dextrose and fructose as a result of the enzymatic activity of the above mentioned Penicillia and *Cladosporium*.

3. Sporulation in *A. aureolatus* is not a light-dependent phenomenon. Colonies grown in the dark even show a better sporulation than in light.

4. When growing on Czapek's solution agar with, as sole source of C, a carbohydrate that *A. aureolatus* can not utilise completely, colonies of the fungus present an evident zonation even when developed in continuous dark. This fact denotes an endogenous cycle, in which rythm has a value equal to the diurnal periodicity.

(Institute for Biological Research, Belgrade, Plant Physiology).

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Rezime

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MORFOGENETSKE REAKCIJE *ASPERGILLUS AUREOLATUS* MUNT.-CVET. & BATA NA RAZLIČITE UGLJENE HIDRATE I SVETLOST

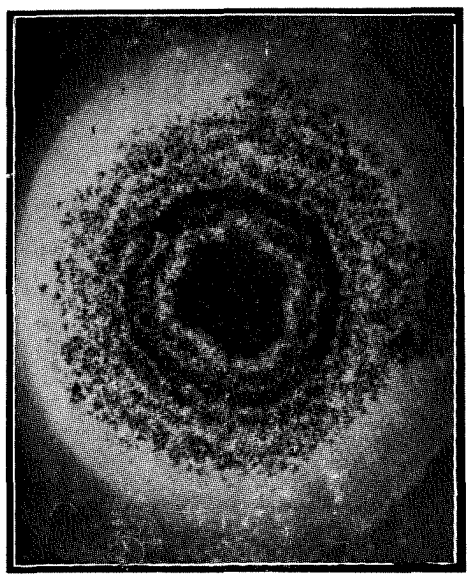
1. Sposobnost *A. aureolatus* da koristi ugljene hidrate kao jedini izvor C varira prema prirodi šećera: heksoze se koriste lako, ali ne i disaharidi (naročito laktoza). Kolonije gajene na agaru sa Čapekovim rastvorom i nekom heksozom se znatno razlikuju od onih na istom medijumu sa saharozom ili laktozom kao izvorom C.

2. Neki organizmi (među kojima su *Penicillium implicatum, P. notatum, Cladosporium* sp.) imaju sposobnost da hidrolizuju saharozu iz agara sa Čapekovim rastvorom i da pojačavaju obrazovanje kondijalnih struktura i proizvodnju žuto-narandžastog pigmenta u imerznim hifama *A. aureolatus*, kada se gaje u istom sudu. Ekstrakti medijuma na kome su rasli ovi organizmi su takođe aktivni u stimulaciji sporulacije i formiranja pigmenta na kolonijama *A. aureolatus*, koje rastu na agaru sa Čapekovim rastvorom sa 3% saharozom ili laktozom. Hromatografska i hemijska analiza ovih ekstrakta su pokazale prisustvo dekstroze i fruktoze, što je rezultat enzimatične aktivnosti pomenutih Penicillia i *Cladosporium*.

3. Sporulacija *A. aureolatus* nije fenomen koji zavisi od svetlosti. Kolonije koje rastu u mraku pokazuju čak i jaču sporulaciju nego na svetlosti.

4. Kada rastu na Čapekovom rastvoru sa ugljenim hidratom koji ne mogu potpuno da iskoriste, kolonije *A. aureolatus* pokazuju jasnu zonaciju, čak i u kontinuelnom mraku. Ova činjenica otkriva endogeni ciklus, čija se ritmika podudara sa dnevnim periodicitetom.

(Iz Instituta za biološka istraživanja, Beograd)



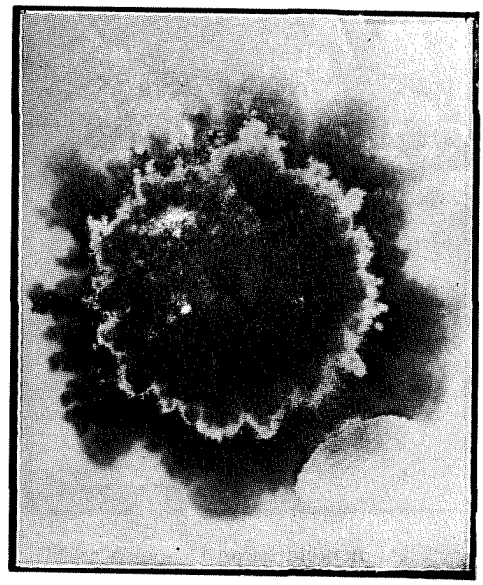
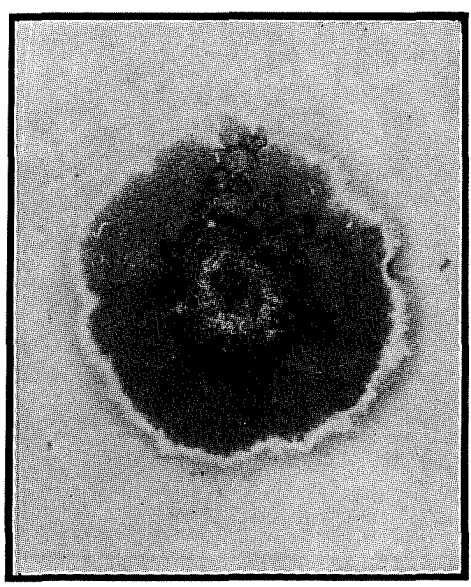


Fig. 1. *A. aureolatus,* a 2 weeks old colony on Czapek's sol. agar + 3% sucrose.
Fig. 2. *A. aureolatus* on PDA, incubation 20 days.



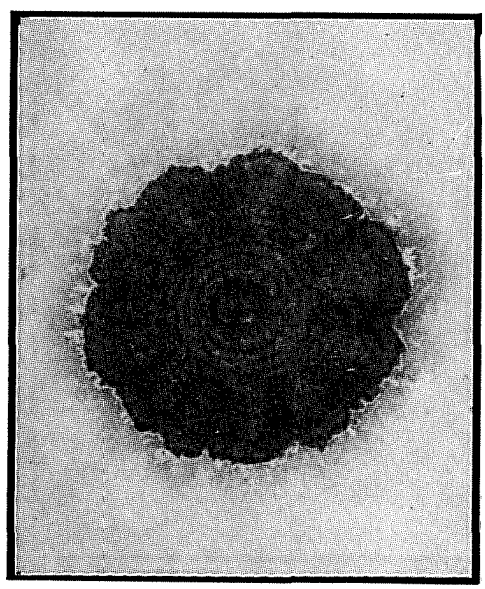
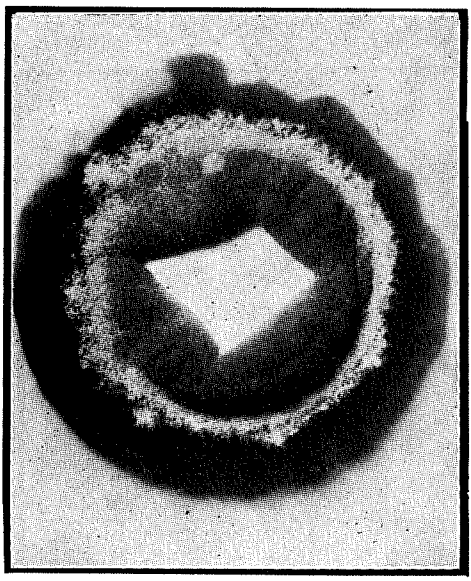


Fig. 3. *A. aureolatus* on malt agar incubated for 20 days in darkness.
Fig. 4. *A. aureolatus on* malt agar, a 20 days old colony submitted to a daily treatment of 12 h white light (1.500 lx), 12 h darkness.



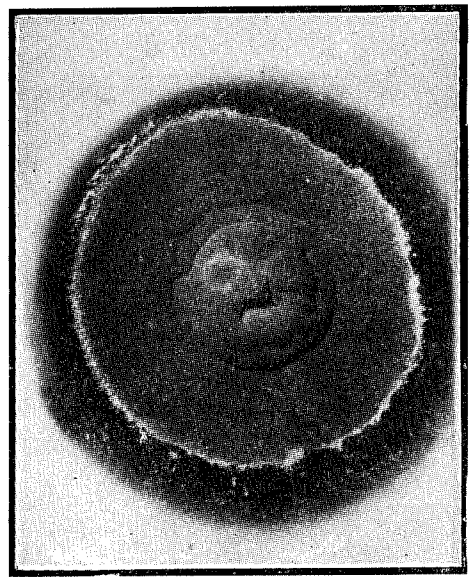
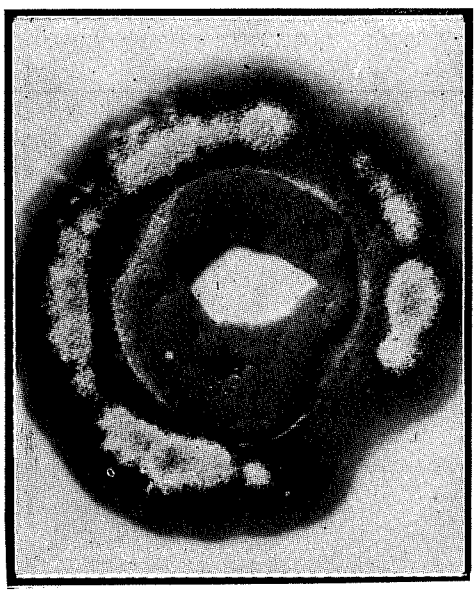


Fig. 5. *A. aureolatus* on Czapek's agar + 10% dextrose instead of sucrose, incubated for 20 days in continuous darkness.

Fig. 6. *A. aureolatus* on Czapek's agar + 10% dextrose instead of sucrose, a 20 days old colony submitted to a daily treatment of 12 h white light (1.500 lx) and 12 h darkness.



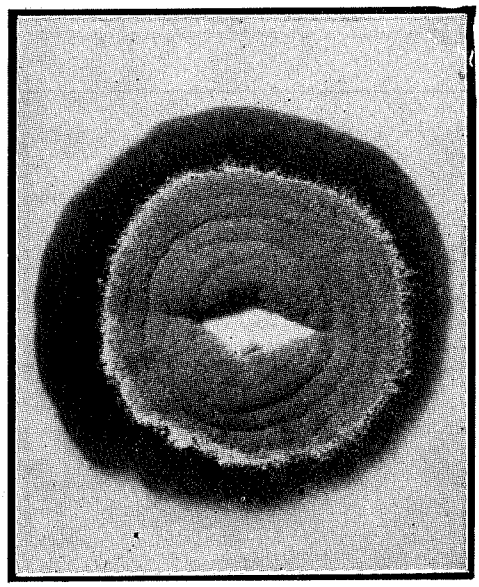


Fig. 7. *A. aureolatus* on Czapek's agar + 10% mannose instead of sucrose, incubation 20 days in darkness.

Fig. 8. *A. aureolatus* on Czapek's agar + 10% mannose instead of sucrose, a 20 days old colony submitted to a daily treatment of 12 h white light (1.500 lx) and 12 h darkness.

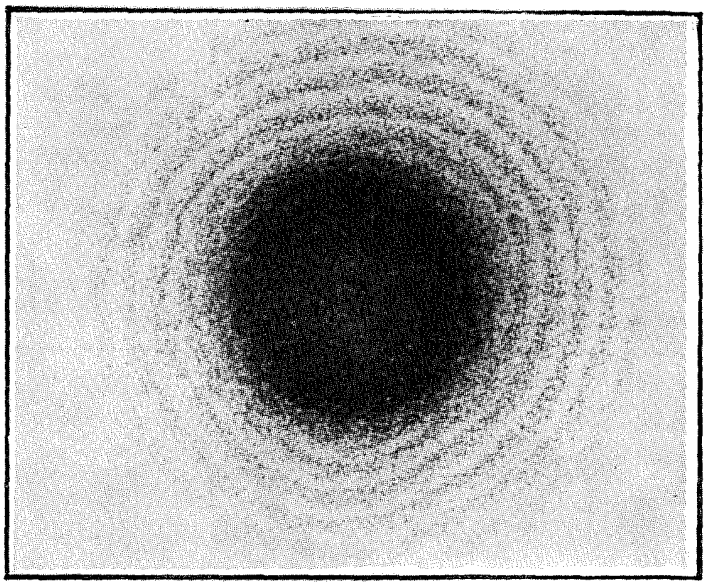


Fig. 9. *A. aureolatus* on Czapek's agar + 10% lactose instead of sucrose, a 20 days old colony incubated in continuous darkness. Note the conspicuous zonations.

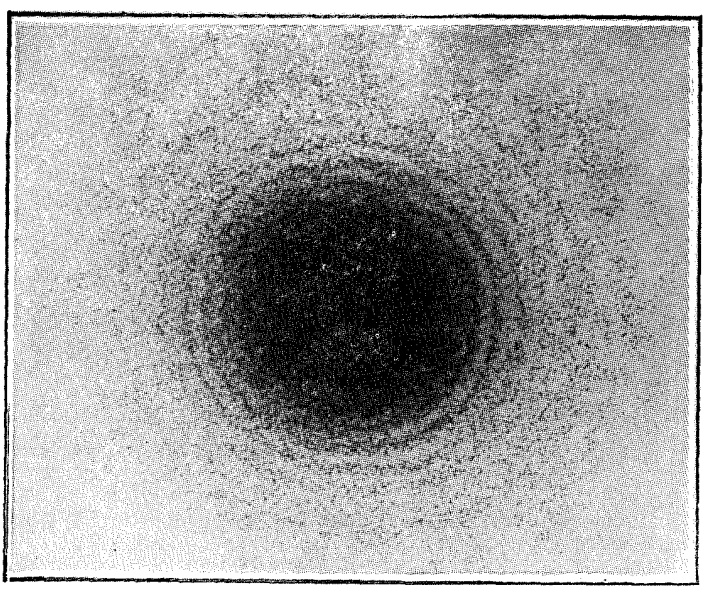


Fig. 10. *A. aureolatus* on Czapek's agar + 10% lactose instead of sucrose, a 20 days old colony submitted to a daily treatment of 12 h white light (1.500 lx) and 12 h darkness.

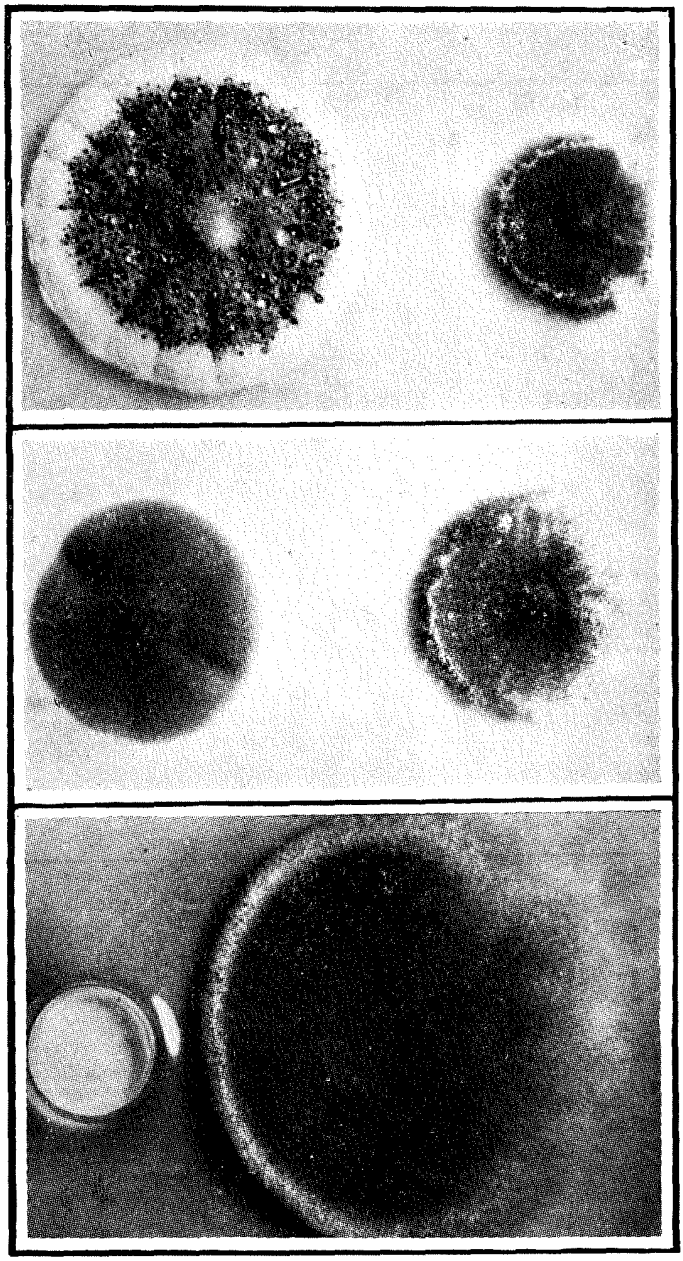


Fig. 11. The influence of *Penicillium notatum* on sporulation and pigment formation of *A. aureolatus* when growing in the same plate on Czapek's agar + 3% sucrose.

Fig. 12. *Cladosporium* sp. and *A. aureolatus* growing in the same plate on Czapek's agar + 3% sucrose; note the effect of the former (left) on the half of the colony of *A. aureolatus* (right) facing to it.

Fig. 13. Sporulation and pigment formation in *A. aureolatus* growing on Czapek's agar + 3% sucrose are enhanced in the half section of the colony facing to a hollow made in the agar and filled with aqueous extract of the agar surrounding a colony of *P. implicatum* cultivated on Czapek's agar + 3% sucrose.

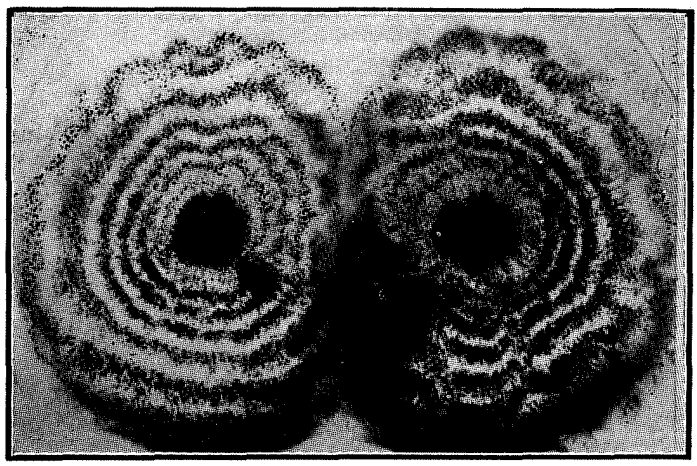


Fig. 14. Two colonies of *A. aurolatus* on Czapek's agar + 3% sucrose, cultivated for 15 days in continuous dark.

Fig. 15. Marginal area of a 25 days old colony of *A. aureolatus* on PDA cultivated in continuous darkness.