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A Cladosarum-like spontaneous mutant of Aspergillus aureolatus

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Summary. A spontaneous mutant of *A. aureolatus* strain 5-BKZ, which in some respects bears a resemblance to the form described by Yuill & Yuill as *Cladosarum olivaceum* (a mutant of *A. niger*) is here described. Secondary sterigmata, instead of producing conidia, thrust out hyaline elements which function again as secondary sterigmata; a terminal cell can resume the function of a primary sterigmata, and branched long chains of hyaline elements are formed as a result. In the mutant here described the alteration of the normal processes is to be observed not only at the level of the primary and secondary sterigmata, but even at the level of the conidiophores and vesicles, which appear extremely reduced or do not exist at all. Sterigmata-like elements directly born from the mycelial filament give to the organism an aspect which drastically differs from the typical one in *Aspergillus*. Pure cultures of this mutant, labeled 5-BK-22b, are completely white and appear surrounded by the orange halo of immersed hyphae characteristic of the wild species. The physiological behaviour of the mutant is the same as that of the typical species. No segregants have been observed until now.

Since the first isolations of *Aspergillus aureolatus* Munt.-Cvet. & Bata in 1963, we have repeatedly remarked in the strain labeled 5-BKZ (obtained from the air, at Belgrade) white patches small in size (⌀ 0,5—2 mm) and limited in number, overgrowing the green surface of the colonies (Fig. 1). These white patches commonly appear with a rather high frequency in one month old cultures independently of the nutrien media (PDA, Czapek's solution agar, Malt Agar, Oat Agar, etc.); they have been observed even in cultures mantained for 3 years at — 20° C. Another strain, VI—35, isolated 5 years later from deteriorating rubber-coated electric cables at the seashore and whose colonies on PDA differ slightly from those of the strain 5-BKZ, never presented these white patches.

Crude microscopical preparations revealed the presence of very long and branched chains of hyaline cellular elements quite different from those in *Aspergillus*, and rather ressembling the structures of *Oidium* or *Verticillium*.

Cross sections made by the paraffin method, as well as more accurate preparations, clearly revealed the intimate relationship of these abnormal cell elements with the normal conidiophores and vesicles of *A. aureolatus*. Having in mind the studies made by Tatarenko (1959) on parasitism in *Aspergillus*, special attention was paid to any possibility

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of this kind. No filaments involving the fructifications or living inside them, or deteriorated host structures, were ever seen.

Pure cultures of these striking forms were obtained following the methods recommended by Raper & Fennell (1965), i. s.:

1) by touching a sterile, moistened needle to a selected surface;

2) cells were thoroughly suspended in water, and the resulting suspension was subsequently diluted in sterile water blanks in steps of 1 : 10;

3) aliquots of 1 ml from 2—3 selected dilutions were repeatedly streaked across the surface of an agar plate;

4) plates were incubated, and isolations were made from those plates showing a limited number (usually 5) of uniformly separated colonies;

5) following germination, well separated incipient colonies were located with the aid of a microscope; a small agar block wth germinated cell were transplanted to fresh agar plates;

6) reisolations were performed in the same way from the resulting individual colonies.

Completely white colonies of the same pattern as those described in *A. aureolatus* (Muntanjola-Cvetković, Nešković & Ćulafić 1968) were obtained; that is:

a) Colonies on media with dextrose, fructose, or mannose as source of C appear surrounded by a gold-yellow, often lobed or arborescent halo of immersed hyphae; the central area of the colony splits and leaves a large hollow; the following zone is convex and not rarely cerebriform (Figs. 2 and 3).

b) Growth on Czapek's solution agar with 3% sucrose is very thin; colonies are plane and they present the general appearance of a mold suffering from some nutritional defficiency. The yellow pigment is only formed when cultures develop under 20° C, or when another fungus or bacteria able to hydrolise the sucrose of the medium grow in the same plate; then, the half section of the *A. aureolatus* mutant colony facing the other organism is influenced by the products of the hydrolitic processes supplied by the other organism and it presents the characteristic halo as well as a more dense growth. At 25° C and higher temperatures the halo of orange immersed hyphae is never seen, except when a second organism growing in the vecinity supplies C in a suitable form. Chromatographic analysis of the extracts surrounding the colonies, using the same methods as applied in the wild type 5-BKZ of *A. aureolatus* (Muntanjola-Cvetković, Nešković & Ćulafič 1968), failed to reveal the presence of detectible amounts of dextrose or fructose, neither in the colonies presenting the orange halo, nor in those without it (Figs. 4 and 5).

The aspect of the colonies may lead one to think of an albino mutant of the typical *A. aureolatus*. However, this is not the case, as in our mutant conidia seem to be completely absent. In many cases conidiophores, vesicles, and primary sterigmata are those of *A. aureolatus*: the conidiophores rather short, brown, smooth; the vesicles globose or ovate, of the same colour as the conidiophores; primary sterigmata no

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different from the typical culture. However, the secondary sterigmata, instead of producing conidia, thrust out cells which have esentially the morphology of the secondary sterigmata themselves; the same procedure is repeated several times to produce a chain of elongate, hyaline, smooth

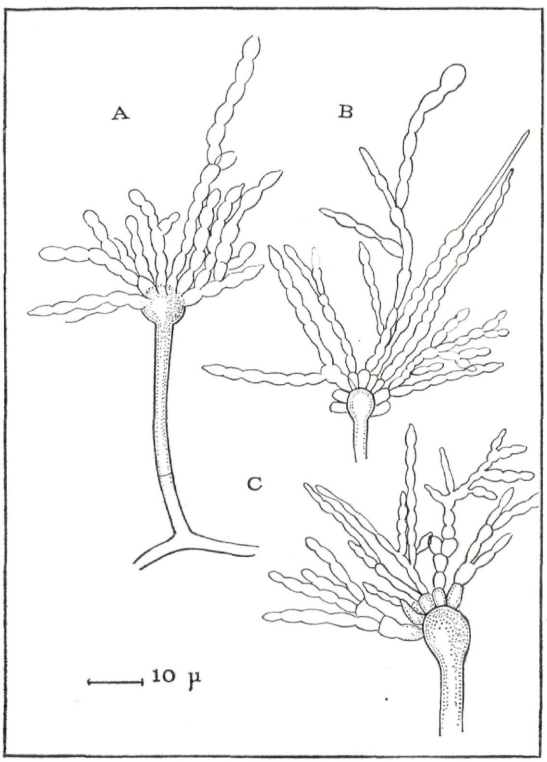

Fig. 6 A—C: Branched chains constituted by elongate, hyaline, smooth elements covering the vesicles of normal, brown conidiophores.

elements. No rarely a terminal cell resumes the function of a primary sterigmata and gives off branched chains of secondary sterigmata. This process can be repeated a large number of times, and long, branched chains of hyaline elements are formed as result (Figs. 6 A—C). A similar aberrant behaviour has been described by Yuill & Yuill (1938)

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for a mutant of *A. niger* which they named *Cladosarum olivaceum*. Occasionally, a terminal cell produces a mycelium-like filament, and this can again thrust out elongate cells which can function as primary or secondary sterigmata.

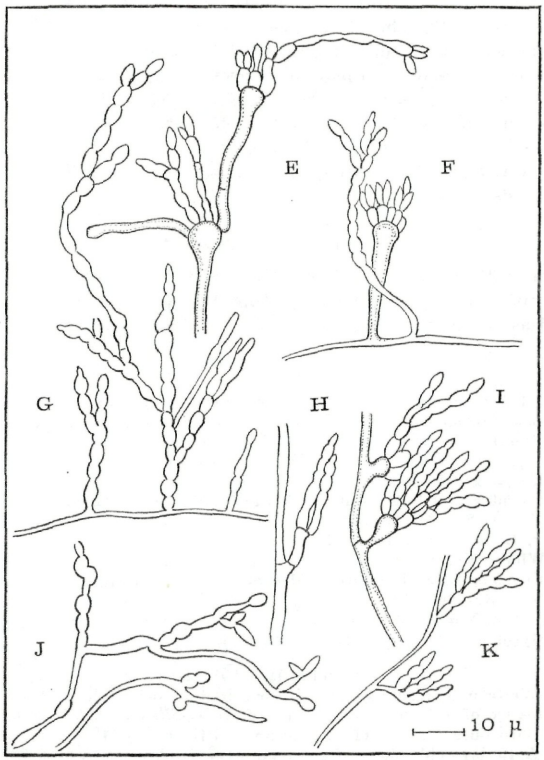

Fig. 7 E—K: Aberrant behaviour of sterigmata-like structures which can be born on extremely reduced conidiophores, or directly from the mycelial filaments.

Sterigmata proliferations, similar in some way to the ones here described, were also observed by B l e u l (1962) in *Aspergillus amstelodami* grown in a strongly alcaline medium. B l e u l interpreted this phenomenon as a disorder in the differentiation at the level of secondary

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sterigmata. In our case the normal processes of differentiation are disturbed not only at the level of the primary and secondary sterimata, but even at the level of the conidiophores and vesicles, which appear extremely reduced or do not exist at all (Figs. 7 E—K). The same hyphal branch may produce well developed conidiophores and branched chains of sterigmata-like elements directly born from the mycelial filament.

Because of the abrupt, marked and persistent differences of the organism here described, and because of its stability, we are led to consider it as a morphological mutant. It grows at the same rate as the wild type and does not need frequent transfers. No segregants have been observed up to now. Cultures of this mutant, labeled 5-BK-22b, have been deposited at the American Type Culture Collection, Rockville, Maryland, U.S.A., and at the Centraal Bureau voor Schimmelcultures — Baarn, Netherlands.

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Plate II and III

Fig. 1. White patches of the spontaneous mutant 5-BK-22b overgrowing the green surface of a 1 month old colony of *Aspergillus aureolatus* on PDA. — Fig. 2. Two pure colonies of the mutant cultivated for 10 days on PDA. — Fig. 3. A 15 days old colony of the mutant on PDA. — Fig. 4. A 20 days old colony of the mutant grown on Czapek's solution agar with 3% sucrose at 19° C. Note the halo of yellow immersed hyphae — Fig. 5. A 20 days old colony of the mutant grown on Czapek's solution agar with 3% sucrose at 27° C. No yellow pigment in the immersed hyphae.

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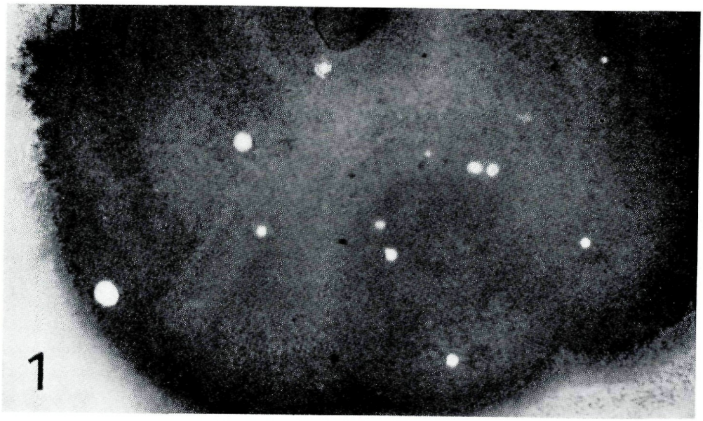

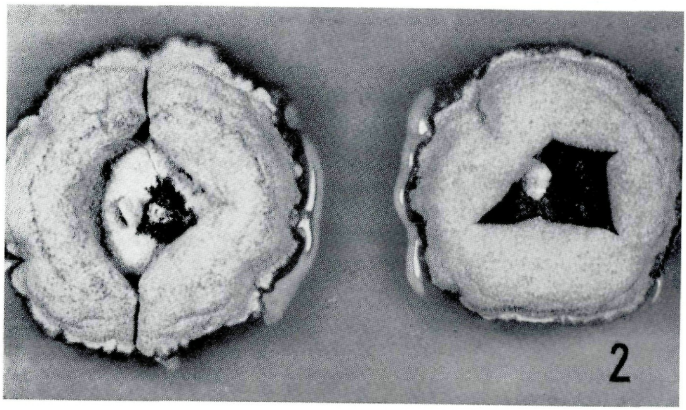

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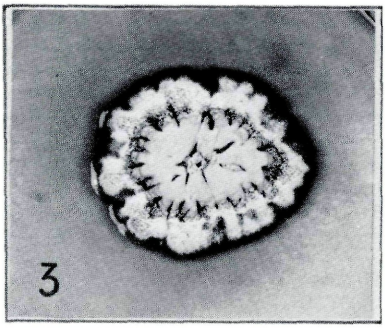

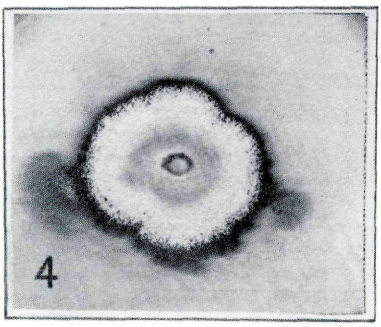

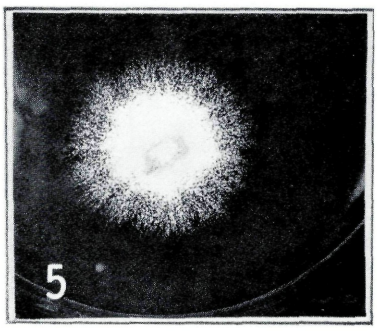

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