MICROBIOLOGICAL ASPECTS OF PENICILLIN

V. Conidiospore Formation in Submerged Cultures of Penicillium notatum[1]

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Received for publication June 12, 1945

Investigation of the conditions of cultivation of the mold *Penicillium notatum* in connection with the formation of penicillin led to the observation that occasionally cultures were obtained with shaking and aeration which, after a certain stage of development, had a greenish cast to them. This type of pigmentation is quite unlike the yellow water-soluble pigment, chrysogenin, commonly formed in cultures of the mold, and apparently is insoluble in the liquid portion of the medium, tending to settle out with the cell material. Microscopic examination of a portion of the pigmented mass revealed that typical conidiospore formation had occurred in the submerged cultures and that the greenish hue of such cultures is due to the grass-green-colored spores of the mold. Their color resembles the spores characteristic of surface cultures. Figures 1 and 2 are photomicrographs of material from typical sporeforming submerged cultures. Figure 1 represents the spore-bearing structures (penicilli) after separation from the clusters of spores, which are shown in figure 2. Figure 3 shows, for comparison, the typical spores and accessory structures in the aerial mycelium of a surface culture of the same strain of *P. notatum*. The characteristic spore apparatus, including sporophores, metulae, sterigmata, and conidiospores, are present in the submerged culture and appear to be essentially the same as those in surface cultures. The long chains of spores are usually absent since they are broken off by the vigorous agitation the culture is subjected to and exist free, singly or in clusters. In many instances in figures 1 and 3 single spores still remain attached to the sterigmata. These probably are incompletely formed spores. However, in figure 1 one chain does appear intact (C).

The formation of spores under submerged conditions by molds belonging to the genus *Penicillium*, and, for that matter, to the class Fungi Imperfecti as a whole, is unique and has not, to our knowledge, hitherto been recorded in the literature. Invariabily, molds of this class which sporulate readily when grown on the surface of solid or liquid media grow profusely under submerged conditions, but the cell material consists only of vegetative mycelium.

Although it is difficult to make an accurate microscopic count because tremendous numbers of spores are trapped in clusters of mycelium, rough counts show as high as 400 million free spores per ml. This figure is low by a con-

[1] This manuscript was ready for publication in May, 1943, but has been withheld from publication owing to its relationship to other material then under U. S. Government secrecy order.

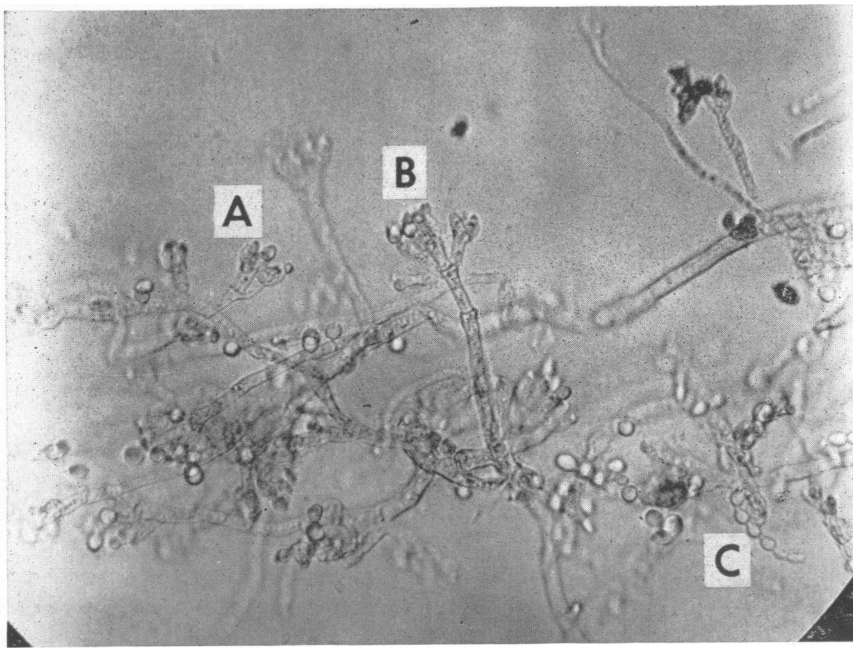

Fig. 1. Spore-bearing Structures in Submerged Growth of P. notatum

Two typical penicilli are in focus at A and B. They show the vegetative mycelium, sporophores, metulae, and sterigmata with single conidiospores attached. These spores probably are incompletely formed. The free spores have been separated for an unobstructed view of the penicilli. An intact chain of spores appears at C. (×530)

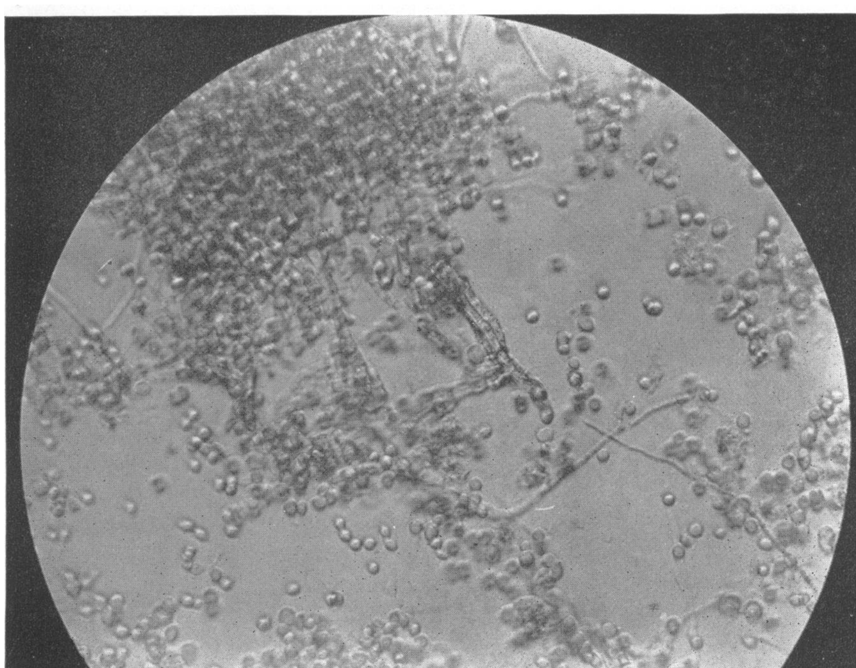

Fig. 2. Masses of Free Conidiospores in Submerged Cultures of Penicillium notatum

Chain formation is broken by the continued agitation. (×530) siderable factor because of uncountable clumps trapped in the vegetative mass. Physiologically the behavior of these spores in respect to growth and penicillin formation is identical with that of surface spores. However, their surface is wetted; and, when used to inoculate surface type liquid cultures, they tend to fall to the bottom, and development of the surface pellicle is retarded. Spores obtained from surface cultures are difficult to wet and float readily on the surface of liquid media, thereby aiding rapid formation of good pellicles. On the other hand, submerged spores are perfectly satisfactory for the growth of *P. notatum* in submerged cultures. They germinate normally and give rise to a profuse, penicillin-producing vegetative mass.

The nature of the medium governs whether spores or only vegetative mycelium will be formed. It is not yet possible to define precisely all the specific factors

FIG. 3. SPORE APPARATUS IN SURFACE CULTURE OF PENICILLIUM NOTATUM. (×530)

which induce the mold to sporulate under submerged conditions. The presence of a rather high concentration of the calcium ion appears to be the most important factor under ordinary laboratory conditions. Calcium chloride at concentrations of 0.5 to 5.0 per cent is most favorable. Abundant sporulation can be obtained in 4 to 6 days at 20 to 30 C in the following practically synthetic medium when aeration and agitation are obtained by rotary shaking.

Per liter of tap or distilled water:

20	g sucrose or brown sugar (grade soft no. 13)
6	g $NaNO_3$
1.5	g KH_2PO_4
0.5	g $MgSO_4 \cdot 7H_2O$
25	g $CaCl_2$

The heavy metal nutrition of the organism also is influential to some extent.

In general, cultures which sporulate in this manner make considerably less vegetative development prior to spore formation than do those in which spores are not formed and vegetative mycelium alone results, as, for example, in active penicillin-producing cultures in other types of media. The typical vegetative development occurs for about 3 days, after which spores commence to form and reach maximum numbers in 4 to 6 days at 20 to 30 C. Prolonged incubation induces secondary germination of the submerged spores in variable percentages of the total number present.

With one exception, about 15 strains of *P. notatum* have uniformly yielded spores in this medium. Also, the only strain of *P. chrysogenum* tested sporulated abundantly.