

The Effect of Bacterial Contaminations Upon the Subsequent Growth of Fungi in the Same Medium

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The following investigation is the outgrowth of a laboratory accident. Using a Difco product, an assistant made a liter of potato dextrose agar. He failed to sterilize it immediately and permitted it to stand for 48 hours at room temperature in the unsterilized condition. When the medium was remembered eventually, it was found to be contaminated by a soft dirty white viscid bacterial growth that had developed on the surface at the point of contact between the medium and the glass of the flask.

Inasmuch as the medium was not to be used for any critical work, it was decided to sterilize it for thirty minutes and to use it in plates and tubes for the purpose of multiplying certain fungus cultures. Very much to our surprise, several species of fungi transferred to this contaminated, but sterilized medium, failed to grow.

We made an attempt to repeat the sequence of phenomena under controlled conditions. Since we had not anticipated that the bacterial contaminant would have any unusual powers, we had not isolated or saved it.

Potato dextrose agar was prepared according to the previous formula. The medium was permitted to stand unsterilized in cotton-stoppered flasks on laboratory shelves for 48 hours. At the conclusion of that period, a bacterial growth that seemed to be similar to the one in the previous accidental experiment appeared on the surface of the agar. As previously, this medium was sterilized for thirty minutes at fifteen pounds pressure. A liter of fresh uncontaminated potato dextrose agar was subjected to the same period of sterilization. The latter preparation was considered a check. Following the sterilization of the contaminated medium, the bacterial growth was no longer visible, and, since there was no subsequent growth of the contaminating organism, it was apparent that this agar was now sterile. The potato dextrose agar that had supported the bacterial growth was slightly darker and a trifle more cloudy than the check; otherwise, there was no apparent difference.

For the purpose of simplicity in the recording of results, the potato dextrose agar which was contaminated and then sterilized will be known as medium "A". The uncontaminated sterilized potato dextrose agar will be known as medium "B".

A transfer culture was made of the bacterial growth on medium "A" previous to sterilization and the organism was determined to be a member of the *B. subtilis* group. This organism was used as a 'staling' organism in all the subsequent experiments reported in this paper.

A series of plates was poured from each medium. The media were inoculated at the center of each plate with either *Diplodia zeae* or *Fusarium moniliforme*. Daily growth was indicated by ringing. Both

F. moniliforme and *D. zeae* covered the plates (90 mm. plates) of normal medium within 100 hours of inoculation, (Medium "B"). The growth made by *F. moniliforme* on medium "A", after the same amount of time had elapsed, was 20 mm., and, although the plates were permitted to stand until the agar had completely dried, no further growth was recorded. The total amount of growth made in medium "A" by *D. zeae* averaged a diameter measurement of only 8 mm. The growth made by both fungi on the uncontaminated medium was normal in every respect with a considerable development of aerial mycelium. The mycelial development in medium "A" exhibited few aerial hyphae and the individual hyphae were distorted, which is an indication of such sharp inhibition as is produced by staling.

In order to determine whether the bacterial contaminant was directly inhibitory to fungus growth, plates were poured with normal potato dextrose agar and inoculated in the center with either *F. moniliforme* or *D. zeae*. After an interval of 24 hours, in which time the fungus was well established on the medium, the bacterial contaminant was also planted near the periphery of the plate in which the fungus had begun growth. After another 24-hour interval, both fungus species exhibited the phenomena associated with inhibition and by 72 hours were sharply inhibited.

Cook¹ studied the results of fungi that succeeded each other in the same culture medium. He used a liquid synthetic medium, 'Cook's No. II liquid medium.' A mixture of this with a medium that had supported growth or a medium in which the fungus previously grown was made one of the constituents was used as test media. He also filtered out the fungus elements of growth, and this liquid was used instead of distilled water in making a new medium. On such media he grew a succession of fungi. In the medium made with the liquid from the filtered growth, he observed acceleration of growth. From this fact, Cook concluded that, if toxic substances were produced by fungus growth, they were not thermostable and were removed when the media were sterilized. He apparently ignored the fact that he was increasing the nutrient content of the medium by the methods that he used and that this increase might account for stimulated growth and might overcome the effect of any toxic inhibitory materials.

Our tests, which were repeated many times, indicate:

1. That a bacterial organism closely related to *B. subtilis* is capable of producing products in potato dextrose agar that are sharply inhibitory to both *Fusarium moniliforme* and *Diplodia zeae*.
2. That the materials so produced are stable to temperatures as high as 250° F. maintained over a period of 30 minutes.
3. That media that have been contaminated by certain bacterial organisms are incapable of supporting the normal development of some fungi. It is probably unsafe to use sterile media that have previously been contaminated in any critical studies with fungi.
4. Fungus-bacterial combinations are abnormal in probably every instance.

¹Cook, Melville T., 1924. Succession of fungi on culture media. Amer. Journ. Bot. 11:94-99.