

The Utilization of Staled Media by Fungi

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The term "staling" was suggested by Dr. Wm. Brown, of the University of London, to describe the condition of a medium that could no longer support the growth of a fungus in a normal manner. A medium was "stale" according to Brown when by-products of fungus growth accumulated to such an extent that further fungus development was inhibited. This occurs eventually whether the same or a different fungus is cultured in a stale medium.

Staling products produced by one organism may inhibit another organism that is growing in the same medium. Thus, Dr. Fleming discovered quite accidentally that *Staphylococcus aureus* was inhibited by the growth products of *Penicillium notatum*. There has existed a difference of opinion as to the cause of staling. It is fairly evident that a medium may become stale for various reasons. In the *Staphylococcus aureus*-*Penicillium notatum* reaction it is quite obvious that the metabolic product, penicillin, is the cause of staling and the consequent inhibitory action. Other metabolic products, as well as unfavorable pH reactions, unfavorable concentrations, or the exhaustion of certain essential nutrients from the medium, might cause staling.

The author has spent years in the investigation of the phenomena associated with staling and numerous reports have been made to the Academy. The research presented here is a continuation of research previously reported.

How stale are unsterilized media after they have been exposed at room temperatures for 24, 72, 120 and 240 hours. The studies here reported give at least a partial answer.

The medium used in this investigation was potato dextrose agar, made from an infusion from two hundred grams of potatoes with twenty grams of dextrose and 15 grams of agar added for each one thousand cc. of distilled water. The final pH of this medium is 5.6. After this medium was prepared in accordance with the formula it was tubed and the tubes were plugged with cotton. The tubes of media were divided into several lots. Lot 1 was sterilized immediately. Lot 2 was permitted to remain at room temperatures for 24 hours before it was sterilized. Seventy-two hours elapsed before lot 3 was sterilized. Lot 4 remained at room temperatures 120 hours before sterilization. Two hundred forty hours was the pre-sterilization period for lot 5. At the close of these various periods of time each lot was sterilized in the autoclave at fifteen pounds pressure for twenty minutes. These various media were dispensed to sterilized petri plates and after the media were hardened, the surface of each medium was streaked with *Staphylococcus aureus* or *Penicillium notatum*.

The organisms used to test the staleness of the various media were

chosen because of the wide publicity that has been given to them recently and because this work with staled media fits into a pattern of experiments that we are making with these test organisms.

Following 24 hours of chance exposure the potato dextrose agar showed little visible change. It was still unclouded and there was no visible fungus growth. There were a few small and submerged colonies of bacteria and there were a few bubbles in the medium indicating the presence of some gas-forming organisms.

The medium exposed 72 hours exhibited quite clearly the presence of gas-forming bacteria. *Penicillia* and some other filamentous fungi were in evidence on the surfaces of the media in the tubes. Medium was cloudy.

After 120 hours of exposure there was a definite fungus mat on top of the medium in each tube, and the medium was cloudy.

The medium exposed for ten days (240 hours) had been host to a succession of fungi and bacteria, beginning with deep gas-forming bacterial colonies, followed by surface growths of *Penicillium* and *Aspergillus*, and miscellaneous organisms that formed a definite fungus mat at the surface. There later developed on this mat a vigorous growth of *Rhizopus* and *Mucor*. The medium was cloudy and filled with granular deposits. There was so much debris in the 240-hour tubes that it was necessary to filter the medium before autoclaving.

Colorimetric pH readings were taken of the various media. The results of these reading follow: 24 hours exposure—unchanged (5.6); 72 hours exposure, 5; 120 hours exposure, 4; 240 hours exposure, 3.

Results of growth on the various media were as follows:

Length of exposure	Growth of <i>P. notatum</i>	Growth of <i>Staph. aureus</i>
None (check)	vigorous	normal
24 hours	"	"
72 hours	"	definitely inhibited
120 hours	"	no growth
240 hours	"	

The medium staled by 240 hours of contamination was so acid that after it had been sterilized it remained in a liquid condition even at low temperatures. This liquid was inoculated with *Penicillium notatum*, but the cloudy, soupy condition made it impossible to judge whether it would support a growth of *Staphylococcus aureus*.

Thus far in this investigation the media have been staled by accidental contaminations. Since there are so many possible explanations of the cause of staling, chance contaminations increase the unknown factors considerably. In order to eliminate some of the uncertainties, sterilized potato dextrose agar was inoculated with *Bacillus brevis*. This organism made a satisfactory growth on the surface of the agar in the inoculated tubes. Nine-six hours following the inoculation of potato dextrose agar with *B. brevis* these tubes of agar were sterilized and the sterilized agar was inoculated in plates with *Penicillium notatum* or with *Staphylococcus aureus*. *P. notatum* grew uninhibited but *Staph. aureus* made no growth. *B. brevis* was selected for this phase of the

investigation because *B. brevis*, a soil organism, is one of the bacteria much used recently in the production of antibiotic substances.

The research here recorded justifies the following conclusions:

1. *Penicillium notatum* is apparently unaffected by a staled medium.
2. *Staphylococcus aureus* is quite sensitive to staling products.
3. A staled medium becomes progressively more acid. The lowering of the pH affects the growth of most bacteria.
4. *Bacillus brevis* serving as the staling agent affected the growth of the two test organisms in the same manner that growth had been affected by chance contaminating organisms.
5. A medium remaining unsterilized is not a medium that will support the normal development of bacteria, nor probably of most fungi.