THE USE OF FRAGMENTED MYCELIAL INOCULUM IN THE CULTURE OF FUNGI¹

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Received for publication December 6, 1946

Savage and Vander Brook (J. Bact., 52, 385) have recently reported the use of fragmented mycelia of *Penicillium notatum* NRRL 832 and *Penicillium chrysogenum* NRRL 1951 as inocula in the production of penicillin. They found that

FIG. 1. APPARATUS FOR ASEPTIC MINCING AND TRANSFER OF MYCELIAL INOCULA

A. Four-L pyrex culture bottle. B. Duraglass fruit jar mounted on Waring "blendor." C. Pyrex aspirator bottle. The culture is grown in bottle A, air being introduced at E through filter F. Tube H is wrapped with cotton. The remainder of the apparatus is sterilized with tubes J, K, I, and P disconnected and wrapped with cotton. After assembly as shown, tube E is disconnected from the air line, tube N attached to an aspirator, clamp O closed, and jar B half filled by inverting bottle A. After this sample is minced, the suspension is drained into bottle C by inverting jar B. The operation may then be repeated. Tube K is then clamped and disconnected. The minced inoculum can be drained and dispensed through tube P.

mycelium minced in a Waring "blendor" and diluted as much as 1:40,000 times adequately substituted for untreated mycelium used at a 1:10 dilution.

We wish to report our experience in the use of a similar technique with other species of fungi and the development of simple equipment for handling the minced mycelium aseptically. Material so prepared has been found satis-

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factory for use in shaker flask experiments and as inoculum for large batches of media.

When a substantial quantity of minced mycelium was required, the equipment shown in figure 1 was employed. The mincer consisted of a Waring "blendor" on which the normal vessel was replaced by a one-quart "Duraglass" fruit jar cut to hold the blending blades. This jar was connected to a culture bottle containing a well-developed mycelial culture of the desired organism. The mycelium was passed into the blender jar in 200- to 300-ml lots, minced one minute, and passed out into a dispensing vessel. In this way substantial quantities of mycelial inoculum could be prepared aseptically in a short time.

The advantages of minced mycelial inoculum over spore inoculum are several. In laboratory studies employing liquid media, better agreement between replicates is obtained, the lag period in initiation of growth is shortened, and it is possible to work with asporogenous species or strains, or with organisms that sporulate poorly. In mass liquid cultures of *Helminthosporium oryzae* Breda de Haan the replacement of spore inoculum by 1 per cent minced inoculum resulted in a twofold increase in the yield of mycelium in two-thirds the time. Fragmented mycelium may well find a place as inoculum in industrial fermentations using fungi.

Minced mycelium has similarly been found suitable for shaker flask experiments and respiration studies. In this case the mincing apparatus can be smaller and has consisted of a small stainless steel cup fitting on the "blendor" base. It differed from that described by Hoffstadt and Tripi (J. Bact., 50, 675) only in having a detachable base.

By mincing for one minute 50 ml of a 5- to 6-day-old mycelial culture of *Gibberella zeae* (Schw.) Petch grown in a shaker flask, approximately 1 to 1.5×10^6 mycelial fragments per ml were obtained. The fragments were freed of media by centrifuging and washing, using aseptic techniques. When examined microscopically, they were found to consist of 3- to 10-cell units, wholly viable and capable of germination at many points. Inoculation with 2 per cent of such material gave maximum mycelium production. This is far more than that found adequate by Savage and Vander Brook, but their report indicates that their culture was much heavier than ours.