Mass Propagation of Conidia from Several Aspergillus and Penicillium Species

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A rapid technique is described for propagation, harvest, and purification of gram quantities of conidia of five Aspergillus and six Penicillium species.

Mass propagation and purification of fungal spores are necessary for many areas of microbiological and biochemical research. Preparing artificial agar media for conidia production is time consuming and expensive based on spore yields (2, 4). The production of spores in submerged culture usually requires a study of different complex media to obtain good sporulation by an organism (5). Natural substrates consisting of cereal grains represent inexpensive media for mass production of spores of various fungi (2-4). Dry-weight yields have been reported to be as high as 1.8 g of conidia/200 g of "pot" barley for Aspergillus ochraceus and Septomyxa affinis (3) and 1.7 g of conidia/200 g of wheat bran for Hemispora stellata (2).

The bread culture technique of Hussong and Hammer (1) was modified so that conidia of Aspergillus and Penicillium could be produced in gram quantities (dry weight). Spores of these species were needed for chemical studies, mass inocula for solid and liquid substrate fermentations, determinations of mycotoxins in spores, and surveying fungal species for the presence of mycoviruses.

Whole loaves of white bread containing no preservatives were purchased at a local bakery and cut into 1.5-cm cubes, 200 g (fresh weight) of which were placed in each 2.8-liter Fernbach flask. The flasks were capped with milk filters and autoclaved for 15 min at 121 C. The flasks were covered with aluminum foil immediately after autoclaving to retain moisture. After the flasks cooled, the bread was inoculated with either 20 ml of a spore suspension or a bread cube from a previous culture plus 20 ml of sterile water. Cultures were incubated at 28 C for 10 days. Flasks were shaken once daily to prevent cube clumping. Conidia were harvested by adding 800 ml of a 10⁻²% solution of Triton X-100 to each flask. Flasks were allowed to stand for 15 to 20 min before they were shaken to dislodge conidia. The bread was separated from the conidial suspension by filtering first through a double layer of cheesecloth and then through glass wool to remove any bread fines. Spores were removed from suspension by centrifugation at 2,500 x g for 5 min. Spore pellets were resuspended in distilled water and centrifuged again. This washing step was repeated twice. After final pelleting, the tared centrifuge containers were turned upside down on paper toweling for 5 to 10 min.

Spore pellets were resuspended in distilled water to give a final concentration of 4 g (wt weight)/10 ml of suspension. For dry-weight determinations, 1-ml samples were pipetted into tared test tubes and dried to a constant weight at 100 C. Spore counts were made using a Petroff-Haussser bacteria counter.

A list of the organisms and their yields of conidia are given in Table 1. All strains grew well, and after a 10-day incubation completely covered the bread cubes (Fig. 1). Conidia preparations of the isolates were found to be viable and were used as stock suspensions for inoculating a variety of natural, semisynthetic, and synthetic media.

A comparison of the few reported conidia yields (3) with our productions indicated a four- to fivefold increase in wet weight and a three- to fourfold increase in dry weight for A. ochraceus. Production studies for Aspergillus niger and Penicillium chrysogenum reported only the number of conidia per unit weight of barley. Lack of wet-weight or dry-weight data makes comparison of actual production impossible. Because Hussong and Hammer (1) grew their organisms on bread, dried the cubes, and ground them into a fine powder, no comparison with their yields was possible.

Microscopy examination (20X) of inoculated bread cubes revealed that the organisms grew over the complete surface, including the pores.
TABLE 1. Conidia yields of Aspergillus and Penicillium species grown on cubed white bread

| Organism         | NRRL no. | Wet weight of conidia/ flask (g) | Dry weight of conidia/ flask (g) | Total no. of conidia/ flask |
|------------------|----------|---------------------------------|---------------------------------|-----------------------------|
| Aspergillus flavus | 3357     | 26.0                            | 8.2                             | 9.8 x 10^10                |
| A. foetidus       | 5265     | 13.0                            | 3.6                             | 1.1 x 10^11                |
| A. niger          | 5266     | 13.0                            | 3.0                             | 9.8 x 10^10                |
| A. ochraceus      | 3174     | 30.0                            | 7.5                             | 3.8 x 10^11                |
| A. parasiticus    | 2999     | 20.0                            | 6.0                             | 2.7 x 10^11                |
| Penicillium brevicompactum | 5260 | 8.1                             | 2.2                             | 1.4 x 10^10                |
| P. chrysogenum Q-176 | 8.0     | 3.6                             | 6.2 x 10^11                    |
| P. palitans       | 3468     | 21.0                            | 6.9                             | 4.2 x 10^11                |
| P. stoloniferum   | 859      | 10.0                            | 3.9                             | 4.7 x 10^10                |
| P. stoloniferum   | 5267     | 11.3                            | 3.4                             | 8.2 x 10^10                |
| P. viridicatum    |          | 14.2                            | 2.8                             | 5.6 x 10^10                |

* Average of 59 isolates.

made by gases during baking. Because of its porosity, bread provides a large surface area for growth and sporulation of organisms. Better aeration is possible than with barley and may account for our apparently higher yields.

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