Simple Method for the Separation of Ascospores

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A simple method for the separation of ascospores is described. To isolate single spores from adhesive ascospores and the mycelium, the suspension was sucked through a combination of sintered-glass plates with different pore sizes.

It is sometimes difficult to determine the number of spores of microorganisms because they stick together very tightly. This problem was dealt with in studies of the ascomycete Byssochlamys fulva, the heat-resistant spores of which are responsible for considerable losses in the fruit juice industry (1, 2). The aggregation of spores makes them unsuitable for counting cell numbers and thus survival curves determined by conventional plate-count methods are inaccurate. An attempt has therefore been made to find a simple method for the solution of this problem. To avoid contamination, all processes have been carried out in an inoculation chamber. The strain of B. fulva used in our investigation was supplied by the Northern Regional Research Laboratory, Peoria, Ill., with the designation A-3849.

For ascospores, the strain was cultivated on Sabouraud Agar (pH 5.6) for 4 days at 37 C and then was held for 14 days at 20 to 25 C; asci began to grow after 6 to 8 days. The asci of B. fulva were partly embedded in the mycelium. The eight spores were closely packed together, surrounded only by a thin membrane. The entire mycelium was scraped off the culture medium with a sterile spatula and put into a sterile flask. After the addition of approximately 20 glass beads (3 mm in diameter) and of 2 to 5 ml of 0.14 M phosphate buffer (pH 7.0), the flasks were sealed with a rubber stopper and an aluminum cap and shaken for 3 min on a Whirl-mixer (Scientific Industry, International Inc., Ltd., United Kingdom). This process broke the ascii away from the mycelium. The asci were then separated from the mycelium in a sterile suction device (Fig. 1). Sintered-glass plates were chosen instead of sintered-glass beakers because they are easier to clean and any combination of pore sizes is possible. After the separation of the mycelium (sintered-glass plates G8, G9, Schott, and Gen), the asci were centrifuged and concentrated in the flasks. After withdrawal of the excess liquid, the asci were shaken once more on the Whirl-mixer for an extended period of 8 to 10 min, during which the asci were broken up and the ascospores were released. Keeping the whole process as sterile as possible, the solution was sucked through another combination of sintered-glass plates (G1, G2) into sterile flasks, whereby a solution of well-separated spores was obtained.

![Diagram of sintered-glass plates](http://aem.asm.org/)

**Fig. 1. Suction equipment for the separation of fungal spores.**

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**LITERATURE CITED**