Simple Method for Mass Production and Collection of Conidia from *Hemispora stellata*

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A simple method is described for the mass production and collection of conidia from *Hemispora stellata*.

In the biochemical studies of fungal spores, the most burdensome task is the collection of large amounts of pure conidia. Although published reports on the biochemistry of fungi are frequent, few refer to conidia, and in most of these cases (1, 2, 3), the conidia are produced in agar cultures, a method that requires time and effort for the careful collection of small yields of conidia.

The present method, which permits far greater yields of pure conidia with almost no effort, was developed during a study of some of the biochemical aspects of *Hemispora stellata* (F. J. Sala, Ph.D. thesis, Univ. of Oviedo, Leon, Spain; A. Lopez, Ph.D. thesis, Univ. of Oviedo, Leon, Spain). This halophilic fungus produces "dun" in salted cod, an alteration responsible for an important part of the economic losses in the cod industry.

*H. stellata* was supplied by the Centraalbureau voor Schimmelcultures (Baarn, Holland) and was maintained at 25 C on malt agar slants containing 7.5% of NaCl.

For the mass production of conidia, spores are incubated in NaCl-wheat bran medium prepared by thoroughly mixing screened, wheat bran and 4.5% NaCl (100:60, w/v). Screening of bran is done through a 1-mm² wire mesh to remove bran fines which would interfere with conidia collection. The NaCl-wheat bran medium is distributed in 65- to 70-g amounts into 2-liter Erlenmeyer flasks or Roux bottles; the flasks are sealed with cotton plugs and sterilized for 15 min at 2 kg/cm². After sterilization, they are inoculated with a spore suspension in sterile water (ca. 3 to 5 ml of a spore suspension, ca. 3 × 10⁶ conidia per ml for each flask) obtained from 10 to 20 malt agar slants. The inoculum is evenly distributed by mixing the contents of the flask with a flame-sterilized glass rod. Flasks are incubated at 25 C for 3 days. Conidia are harvested by shaking the contents of flasks with a suitable amount of sterile 15 to 20% NaCl. The suspension obtained, containing wheat bran, starch granula, and conidia, is filtered through four layers of sterile, cheese cloth. Conidia from this filtrate are collected by centrifugation for 5 min at 1,000 rev/min in a Martin Christ Universal centrifuge and then at 4,000 rev/min for 5 additional min (non-stop). Three layers are formed in the deposit (Fig. 1): a small upper, white layer of starch granula; a second dark brown layer of fairly pure conidia; and a bottom layer containing bran fines, starch granula, and a small proportion of conidia. Spores from the bottom layer can be removed by centrifugation, as before. After centrifugation, the top, white layer can be easily washed out with the jet of a washing bottle; the medium layer is collected with a spatula, resuspended in sterile water, and purified further by repeating centrifugation until the desired.

![Diagram](https://via.placeholder.com/150)

**Fig. 1. Deposit from centrifugation of conidia suspensions.**
degree of purity is achieved. No more than 2 to 3 centrifugations are usually required.

By this method a pure mass of conidia (400 to 600 mg, dry weight), without hyphal fragments or starch granula, is obtained from each 2-liter Erlenmeyer flask.

These conidia may be used for enzymatic as well as biochemical studies, for germination is not affected even after 30 days of storage in 15 to 20% NaCl at 0 to 5 C.

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