Johnson, T. E. and D. R. Stadler. A method for isolating protoperithecio.

In this method, protoperithecio ore grown for seven days at room temperature on petri plater containing 15 ml of Westergaard's crossing medium with 1.5% agar. After this time, large numbers of protoperithecia are visible. The agar from one plate is cut into squares, and is then immersed in 50 ml of sterile water and ground in a Sorvall Omnimixer at setting nine for one minute. This suspension is diluted into 2 liters of water and stirred continually at room temperature during the rest *of the* procedure. It is important that this suspension be dilute and that clumps of mycelia not be allowed to form.

The suspension is siphoned at a rate of 300 ml/hr into a race tube filled with distilled water. Both ends of this U-shaped tube hove been bent about 30° from the horizontal. The bent portion at one end is slightly longer so that the suspension will flow through the tube and out the lower end without pumping. Most of the mycelial material either flows completely through the tube or collects near the for end, while the protoperithecio tend to collect near the mouth of the tube.

We collect the material found in the first ten cm of the tube and find it to be relatively free of mycelia. A second passage of this material giver protoperithecia almost free of any contaminating mycelia. We have been able to collect as much as one gram (wet wt.) of protoperithecia from four petri plates. The protoperithecia seem not to have suffered too badly from the isolation procedure, as most of them con still serve as foci for vegetative growth and form a distinctive colony type when plated on sorbose *media*. We hove been unable to fertilize these *isolated protoperithecia,* possibly because the *trichogynes* ore removed during the initial process of shearing. We are currently characterizing some of the proteins which are contained in the protoperithecio. -

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