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Storage of monokaryotic strains of Podospora anserina

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Abstract

Maintenance of *Podospora anserina* strains for experimental purposes is very time consuming (see Esser 1969 Neurospora Newsl. 15:27-31) and methods have been published that address this issue by freezing ascospores at -80 oC (Begel and Belcour 1991 Fungal Genet. Newsl. 38:67). Although the latter approach does reduce the amount of time required for yearly sexual crosses and ascospore isolation, it does not resolve the problem of the time required to rapidly generate monokaryotic hyphae, that are needed as a source for inoculum for many types of experiments.

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Brief Notes

Storage of monokaryotic strains of *Podospora anserina*

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Maintenance of *Podospora anserina* strains for experimental purposes is very time consuming (see Esser 1969 Neurospora Newsl. **15**:27-31) and methods have been published that address this issue by freezing ascospores at -80 ^{o}C (Begel and Belcour 1991 Fungal Genet. Newsl. **38**:67). Although the latter approach does reduce the amount of time required for yearly sexual crosses and ascospore isolation, it does not resolve the problem of the time required to rapidly generate monokaryotic hyphae, that are needed as a source for inoculum for many types of experiments. For example, experiments on the phenomena of senescence in this fungus require the isolation of mononucleate ascospores and the rapid generation of large inocula of monokaryotic hyphae for starting liquid batch cultures. In order to obtain monokaryotic strains for *P. anserina*, one has to initiate a sexual cross between opposite mating types and then, once the peritheca start shooting ascospores, six spored asci have to be recovered and analysed for mononucleate ascospores (note only 1- 2% of asci contain find mononucleate ascospores along with the typical binucleate heterokaryotic spores, Esser 1969 Neurospora Newsl. **15**:27-31). Once isolated monokaryotic strains are not very stable and will deteriorate (senescence) even when stored on agar slants at 4 ^{o}C.

Podospora anserina A and S races were used in this study. Sexual crosses were initiated and mononucleate ascospores were recovered and placed on germination corn meal agar plates. The plates were allowed to develop in the dark for 2 days at 28 ^{o}C, then a small agar block was removed from the plate for mating type determination. The remainder of the monokaryotic *Podospora* agar plate/culture was aseptically added to a sterile blending chamber (Dupont Instruments 50 ml chamber) filled with 20 mls of filtered liquid corn meal infusion media (LCMI, 20 g of corn meal/L, Media and general culture conditions were as described in Esser 1969 Neurospora Newsl. **15**: 27-31). The blended material was used for inoculating 1 L of LCMI media (containing 50 Fg of ampicillin/ml). The liquid culture was allowed to develop in the dark for 20 hours at 28 ^{o}C on a rotatory shaker (250 rpm). The mycelium was collected by centrifugation at 10 000 rpms for 15 min in 250 ml centrifuge bottles in a GSA3 rotor. After decanting the liquid media from the centrifugation bottles each mycelium pellet was taken up in 4 mls of storage medium (0.5 M sorbitol/50 mM Tris-HCl, pH 8.0, and 1 ml of 40% (wt/vol) PEG 8000/50 mM Tris-HCl, this storage medium was adapted from the *Neurospora crassa* spheroplast storage medium given in Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. USA **83**:4869-4873). All the dissolved pellets were combined and added to a sterile blending chamber. The mycelium was homogenized with a Omni mixer (Serval, Ivan Serval, Inc.; Norwalk, Conn., USA) at a power setting of 100 for 1 minute. The resulting homogenate was kept on ice and convenient portions (0.5 to 1 ml aliquots) were transferred to sterile microcentrifuge tubes. The tubes were gentle stirred to prevent the hyphal fragments from settling to the bottom of the tubes and then the tubes were placed in a -80 ^{o}C freezer after flash freezing on dry ice (note: no glycerol or DMSO was added to the tubes). These tubes represent a source of inoculum that is readily available anytime an experiment is to be initiated. Ten Fl aliquots are usually taken from partially thawed tubes and transferred onto corn meal agar plates. The aliquot can be spread with a glass rod over the entire plate and a confluent “lawn” of mycelium were obtained within three days. These plates can then be used for inocula of larger liquid batch cultures (one agar plate/2 L). So far, mycelial aliquots from tubes stored up to 12 months have been used and still found to be viable. Using this approach we can store and maintain the individual mating types of the A and S races and quickly generate Petri plate cultures suitable as sources of inocula for liquid batch cultures without the time consuming need to continuously set up crosses and reisolate the individual mating types.

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