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V. E.A. Russo

T. Sommer

J. A.A. Chambers

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Abstract

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Russo, V.E.A., T. Sommer and J.A.A. Chambers

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Our laboratory has been studying the photoinduction of protoperithecia for several years. Originally all work was done with Westergaard and Mitchell medium, but this medium was found to be extremely inconvenient because it is difficult to prepare more concentrated than a 2x stock. Vogel's medium, although it can be prepared as a 50x stock, has a high nitrogen content which favours the production of conidia but not of protoperithecia. The convenience of a 50x stock prompted us to try to modify Vogel's medium for use as a crossing medium.

We found that if the nitrogen concentration of the 50x stock was reduced from 100 gm to 10 gm NH_4NO_3 per liter then the medium promotes the production of enough protoperithecia for crossing and mating type testing.

For colonial growth we replace the sucrose with 1.1% sorbose and 0.1% glucose. To test for protoperithecia production in this medium it was necessary to reduce the nitrogen concentration in the 50x stock to 1 gm of NH_4NO_3 per liter. Sorbose and sucrose are added from a sterile stock of 20% sorbose, 2% glucose that is steamed (90°C, 30 min) twice with a 24h interval. On these plates protoperithecia appear after 4 days in the dark at 23°C. Typically a colony of 1 cm diameter has about 400 protoperithecia. Illumination accelerates the production of protoperithecia.

TABLE I

Ascospore yield end viability from different media (arg-10 , A x wc-l, a)

Medium	Ascospores	%Germination+
Vogel's modified[a]	3.6×10^4	60
"	2.2×10^4	60
"	4.3×10^4	82
Westergaard's	4.0×10^4	81
	4.0×10^4	47
"	4.5×10^4	56

+ After 24h germination at 34°C.
a The 50x stock contained 10 gm NH_4NO_3/liter.
Data from three crosses on each medium.

Plates for testing mating type with a non-fluffy tester are incubated at 23°C for 3 days and conidia cleaned from the edges of the plate with an ethanol-soaked tissue on the third and fourth days. Mycelia can be fertilized between the 4th and 7th days.

For crosses we use an essentially similar procedure in tubes with fertilization between the 5th and 7th days. Ascospores appear after about two weeks. If the female parent is an auxotroph for a potential nitrogen source we use'the minimal concentration compatible with good growth, e.g. 0.3 mg/ml^{-1} arginine for arg-1, -6, -10 and 0.7 mg/ml^{-1} glutamate for am^{132}.

We have been using this system for more than a year without any problems. We have compared the fertility of crosses on nitrogen-poor Vogel's and Westergaard and Mitchell's nineteen days after fertilization. Ejected ascospores were collected, counted and the efficiency of germination determined. We found the two to be comparable (Table I). Detailed experiments on the role of nitrogen in differentiation will be published later:

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