Fungal Genetics Reports

Volume 47 Article 6

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Recommended Citation

Adhvaryu, K. K., and R. Maheshwari (2000) "Use of microconidia for testing the genetic purity of Neurospora stocks," *Fungal Genetics Reports*: Vol. 47, Article 6. https://doi.org/10.4148/1941-4765.1202

Use of microconidia for testing the genetic purity of Neurospora stocks

Abstract

Uninucleate microconidia have been used to check the purity of laboratory stocks of *Neurospora*. Stock cultures of *N. crassa* and *N. intermedia* become heterokaryotic due to spontaneous mutation.

Regular Papers

Use of microconidia for testing the genetic purity of Neurospora stocks

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The Lindegrens (Lindegren and Lindegren 1941 J. Hered. 32: 404-412) stated that "single ascospore cultures of *Neurospora* which are stored or carried in culture for a few months almost invariably become heterokaryotic due to spontaneous mutation". The Oak Ridge wild type *N. crassa* OR23-IV*A* (FGSC 2489) was shown to accumulate spontaneous translocations (Perkins and Kinsey 1993 Fungal Genet. Newsl. 40: 67-68). We demonstrate an easy method of examining the purity of laboratory stocks of *Neurospora*, based on the appearance of morphological variations among single microconidia derived cultures.

N. crassa ORS-6*a* and OR23-IV*A* had been continuously grown in our laboratory for over 10 years. Microconidia were produced by the cellophane method (Pandit and Maheshwari 1993 Fungal Genet. Newsl. 40: 64-65) and plated on sorbose medium (Davis and de Serres 1970 Meth. Enzymol. 17A: 79-143) supplemented with yeast extract (0.05%) and casamino acids (0.05%). After four days at 34°C, 100 colonies were picked on Vogel's minimal medium supplemented with yeast extract (0.05%) and casamino acids (0.05%) and subcultured on minimal medium. No auxotrophs were found. However, morphological variants showing button type, carpet type, or very sparse growth were observed at a frequency up to 3%. The frequency of variants remained the same in the absence of iodoacetate that was included in the medium to stimulate the production of microconidia. Two healthy microconidial-derivatives of ORS-6*a* and OR23-IV*A* were selected and after storage at –20°C for about 1 month, they were again resolved by microconidial plating. Interestingly, morphological variants reappeared at a frequency of 2-3%, suggesting that spontaneous mutations occurred readily in some nuclei or in mitochondria.

Next, we tested the purity of *N. intermedia* species tester strains of *A/a* mating types that we developed in our laboratory for use in the identification of wild isolates from India and as a reference 'wild-type' *N. intermedia* for use in genetic analysis of intraspecies genetic variation. These strains were derived by crossing a f_5 *N. intermedia* from Taiwan (Perkins and Turner 1988 Exp. Mycol. 12: 91-131), with a f_5 *N. intermedia* from Maddur, in India, as given below.

f_5 Shp-1*A* (FGSC3416)×Shp-1*a* (FGSC3417) Maddur 1991-4*a* × Maddur 1991-10*1A*

↓ ↓

f_{10} RM 125-2 *A* × f_{10} RM 126-1 *a*

↓

RM 137-1 *A* × RM 137-2 *a*

↓

f_8 RM 245-3 *A* / RM 245-1 *a*

The *N. intermedia* tester strains RM 245-3*A* and RM 245-1*a* showed good fertility with local isolates, and produced abundant macroconidia and microconidia on appropriate media. These cultures were stored at –20°C and subcultured as and when necessary during a period of about four years after which their purity was examined. Among the 100 microconidial derived cultures, two types of morphology were observed in almost equal numbers in both *A/a* testers: (1) 'conidial' type that resembled the parent; and (2) 'aconidial' type that did not produce blastoconidia or arthroconidia, but were otherwise healthy and formed aerial mycelium. The aconidial culture showed minor constriction budding but major constriction budding was absent (Springer 1993 BioEssays 15: 365-374). Despite both conidial type (normal) and aconidial type (mutant) nuclei being present in equal proportion, the parent cultures were conidial, suggesting that the 'aconidial' mutation was recessive. To examine the genetic nature of aconidial cultures, crosses were made using conidial and aconidial types (Table 1). The appearance of conidial progeny in reciprocal crosses of aconidial × aconidial strains was unexpected, indicating that these two strains had mutations at different loci.

The *N. intermedia* tester strains RM 245-1*a* and RM 245-3*A* were expected to be homokaryotic, but microconidial analysis showed that both contained mutant nuclei. These strains were no longer isogenic and suitable as reference wild-type *N. intermedia*. The fact that morphs were detected in both mating types at the same time indicated the inherent genetic instability of these strains, possibly arising from of a nuclear transposon-like element in them, although there is no evidence for transposons passing through several sexual cycles. Alternatively, the instability of the strains could result from mitochondrial mutations. These experiments demonstrate that microconidia are useful in checking the purity of *Neurospora* stocks from time to time.

Acknowledgement: This work was supported by a grant from Department of Science and Technology, Government of India, to RM.

Table 1: Phenotype of progeny in crosses of microconidial derivatives of *N. intermedia* tester strains[a]

Cross	Progeny analyzed	Conidial	Aconidial
1. ♀ RM 245-3*A*-100 × ♂ RM 245-1*a*-100	44	44	nil
2. ♀ RM 245-3*A*-1 × ♂ RM 245-1*a*-1	44	15	29
3. ♀ RM 245-1*a*-1 × ♂ RM 245-3*A*-1	35	7	28
4. ♀ RM 245-3*A*-1 × ♂ RM 245-1*a*-100	44	23	20
5. ♀ RM 245-1*a*-100 × ♂ RM 245-3*A*-1	21	11	10
6. ♀ RM 245-1*a*-1 × ♂ RM 245-3*A*-100	49	29	20
7. ♀ RM 245-3*A*-100 × ♂ RM 245-1*a*-1	40	15	25

[a]RM 245-3*A*-100 and RM 245-3*A*-1 have conidial and aconidial phenotypes respectively, and were derived by microconidial plating of RM 245-3*A*.

RM 245-1*a*-100 and RM 245-1*a*-1 have conidial and aconidial phenotypes respectively, and were derived by microconidial plating of RM 245-1*a*.