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Abstract

A major difficulty that has limited the use of uninucleate microconidia in genetic research is their low and erratic germination. We found that the supplementation of sorbose plating medium by amino acids, notably aspartic acid and methionine, markedly improved germination and plating efficiency of microconidia of *mcm* and *pe fl* genotypes of *N. crassa*. The plating efficiency of mcm microconidia in amino acid supplemented medium was comparable to macroconidia.

Increase in germination and plating efficiency of *Neurospora crassa* microconidia by amino acid supplementation

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A major difficulty that has limited the use of uninucleate microconidia in genetic research is their low and erratic germination. We found that the supplementation of sorbose plating medium by amino acids, notably aspartic acid and methionine, markedly improved germination and plating efficiency of microconidia of *mcm* and *pe fl* genotypes of *N. crassa*. The plating efficiency of *mcm* microconidia in amino acid supplemented medium was comparable to macroconidia.

N. crassa produces very small numbers of uninucleate microconidia compared to multinucleate macroconidia. If microconidia can be selectively obtained uncontaminated with macroconidia, they would be preferred for use in DNA-mediated transformation, analysis of beterokaryons, and recovery of recessive mutants from wild isolates. This problem is overcome by use of microconidial genotypes or by the use of cellophane culture technique that allows microconidia to be selectively obtained even from wild type strains (Maheshwari 1999 Fungal Genet. Biol. 26: 1-26). However, the problem of erratic or low germination and plating efficiency had remained despite attempts to overcome it (Kalpana *et al*. 1998 Fungal Genet. Newsl. 45: 19). An observation that macroconidia of a histidine-transformant showed an unusual, non-specific requirement of amino acids for optimal germination led us to examine the effect of amino acid supplementation on plating efficiency of microconidia with surprising results.

The *mcm; A* genotype (FGSC #7455) produces microconidia by microcycle conidiation when macroconidia from agar grown cultures are incubated in liquid shake cultures for 24 h (Maheshwari 1991 Exp. Mycol. 15: 346-350) whereas *pe fl; A* (FGSC #3072) and *fl;dn; A* (FGSC #3517) produces microconidia in agar-grown cultures in 6-8 d. Microconidia (200 or 500, based on haemocytometer counting) were spread on sorbose plating medium (Davis and de Serres 1970 Methods Enzymol. 27A: 79-143). The amino acids (Sigma) were added before autoclaving. The plates were kept at 34°C in dark and the colonies were counted on the fourth or the fifth day. The results of *mcm* and *pe fl* are from three platings. Microconidia of *fl; dn* were plated once. Three replicates were used for each plating.

Although we adhered to nearly the same conditions, the plating efficiency of microconidia varied in different platings of the same strain. Our experiments were limited to the strains mentioned above. Addition of 0.1% vitamin-free casamino ac (Difco) markedly improved plating efficiency of both *mcm* and *pe fl* microconidia (Table 1); the colonies appeared earlier (day 2) than in control plates (day 4). Plating efficiency of *fl; dn* microconidia was very low; therefore this strain was not used further. Of the individual amino acid tested, aspartic acid and to a lesser extent, methionine, were most effective. With amino acid supplements, the plating efficiency of *mcm* microconidia was comparable to macroconidia of different genotypes (55-90%) that have been studied in our laboratory. Alanine, cysteine, glutamic acid, serine and tryptophan improved plating efficiency marginally whereas arginine, glycine, lysine, phenylalanine and histidine had no effect. At the concentrations tested, leucine and threonine were inhibitory. The mechanism of stimulation by amino acid is not known but it is clear that they only triggered germination. The results also show that genotype of strain has a marked influence on microconidial germination.

Table 1. The effect of amino acid supplementation on plating efficiency of microconidia[a]

Amino acid	% colonies ± s.d.		
	mcm; A	*pe fl: A*	*fl; dn; A*
Control	20 ± 15	9 ± 5	0.6
Alanine	46 ± 10	17 ± 7	0.2
Arginine	16 ± 9	12 ± 9	0.8
Aspartic acid	65 ± 14	55 ± 11	7
Casamino acid	76 ± 14	32 ± 9	0.7
Cysteine	46 ± 29	44 ± 19	4.5
Glutamic acid	45 ± 10	38 ± 18	2.4
Glycine	21 ± 19	14 ± 8	n.d.
Histidine	32 ± 10	13 ± 7	0.1
Leucine	8 ± 5	7 ± 3	2
Lysine	23 ± 5	29 ± 7	2
Methionine	59 ± 25	55 ± 13	2
Phenyl alanine	31 ± 11	16 ± 7	0.06
Serine	45 ± 9	20 ± 7	0.1
Threonine	2 ± 1	2 ± 2	0.2
Tryptophan	48 ± 4	22 ± 5	0.6
Tyrosine	56 ± 7	15 ± 7	n.d.

[a]Amino acids were used at following concentrations: alanine and glutamic acid at 40 mg/100 ml; arginine, cysteine, glycine, lysine, methionine and serine at 50 mg/100 ml; aspartic acid at 100 mg/100 ml; histidine, leucine, phenylalanine, tyrosine and tryptophan at 20 mg/100 ml; and threonine at 7.5 mg/100 ml. n.d. not determined.