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Nitrogen and carbon starvation regulate conidia and protoperithecia formation in Neurospora crassa grown on solid media

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Abstract

It is known that nitrogen and carbon limitation regulate conidiation in shaken liquid cultures (Guignard et al. 1984 Can. J. Microbiol. 30:1210-1215; Muller and Russo 1989 Fungal Genetics Newsletter 36:58-60), and that nitrogen starvation regulates protoperithecial formation in solid medium (Sommer et al. 1987 Planta 170:205-208).

Nitrogen and carbon starvation regulate conidia and protoperithecia formation in *Neurospora crassa* grown on solid media

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It is known that nitrogen and carbon limitation regulate conidiation in shaken liquid cultures (Guignard et al. 1984 Can. J. Microbiol. *30*:1210-1215; Muller and Russo 1989 Fungal Genetics Newsletter *36*:58-60), and that nitrogen starvation regulates protoperithecial formation in solid medium (Sommer *et al*. 1987 Planta 170:205-208). We investigated the influence of nitrogen and carbon starvation on both sexual and asexual differentiation under exactly the same conditions of growth on solid medium.

We grew *Neurospora crassa* (OR wild type, mt *a*) on 20 ml agar medium in 8 cm diameter petri plates covered with circular dialysis membrane. The medium was a modified Vogel's medium (Russo 1988 J. Photochem. Photobiol. *2*:59-65) with 4 mM NH*4*Cl as sole nitrogen source and 1% (w/v) sorbose plus 0.1% (w/v) glucose as sole carbon sources. The plates were inoculated with 1 x 10 *4* conidia spread onto the dialysis membranes and were incubated in the dark at 23*o*C. After three days the membranes were transferred onto new plates. The four different types of plates were:

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+N +G    (50 mM NH4Cl, 1% sorbose, 0.1% glucose)

+N -G    (50 mM NH4Cl, no carbon source)

-N +G    (no nitrogen source, 1% sorbose, 0.1% glucose)

-N -G    (no nitrogen source, no carbon source)
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All plates were illuminated with 1 min of blue light (6 W/m *2*) just after transfer and 24 h later. Protoperithecia were counted using a binocular microscope 48 h after the transfer (Degli Innocenti and Russo 1983 Photochem. Photobiol. 37:49-51) and macroconidia were collected with 5 ml sterile twice-distilled water. The number of viable macroconidia was determined immediately by plating. The data are shown in Table 1 and analyzed in Table 2.

Table 1. Number of conidia and protoperithecia per plate in nitrogen or glucose starvation

Experiment	+N +G		+N -G		-N +G		-N -G	
	conidia	Pp	conidia	Pp	conidia	Pp	conidia	Pp
I	1.5x107	0	5.0x10^8	1000	3.7x10^4	8950	1.3x10^7	11400
II	1.2X10^7	0	5.3x10^8	200	4.3x10^4	8800	1.7X10^7	10250
III	0.9x10^7	0	1.5x10^8	500	8.0x10^4	13150	1.3x10^7	10050
Average	1.2x107	0	4.0x10^8	550	5.3x10^4	10300	1.3x10^7	10550

Pp = protoperithecia

In each experiment, the data are the average of three plates.

Table 2. Regulation of conidia and protoperithecia by nitrogen and carbon starvation. Analysis of data of Table 1.

	conidia	protoperithecia
(-N/+N) +G	(5.3X10^4/1.2X10^7)=0.005	(10,300/0)>10,300
(-N/+N) -G	(1.3X10^7/4.0X10^8)=0.03	(10,550/570)=18
(-G/+G) +N	(4.0X10^8/1.2X10^7)=30	(570/0)> 570
(-G/+G) -N	(1.3X10^7/5.3X10^4)=260	(10,550/10,300)=1

(-N/+N) +G is the ratio between the number of conidia (or protoperithecia) in plates containing no nitrogen and the number of conidia (or protoperithecia) in plates containing nitrogen. In both plates carbon was present. The other symbols have similar meanings.

Nitrogen starvation inhibited production of conidia and induced production of protoperithecia. Carbon starvation induced both conidia and protoperithecia. In the case -N(-G/+G) the ratio was 1, but this is probably due to saturation of the biological system. It was not possible for cultures to make more than 10,000 protoperithecia/plate and this value is reached already by nitrogen starvation.

Nitrogen and glucose acted synergistically for conidial production. For example, the plates of +N-G type had 1 x 10 *4* more conidia than the plates of -N+G type. It would be interesting to know whether glucose and nitrogen starvation influenced the expression of con and bli genes. *con* genes are candidates as important genes in the conidiation process (Berlin and Yanofsky 1985 Mol. Cell. Biol. 5:849-855) while bli genes are candidates as important genes in the formation of protoperithecia (Sommer et al. 1989 NAR 17:5713-5723).

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