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Abstract
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It is a general belief that in a liquid shaker culture, wild-type Neurospora crassa exhibits only vegetative growth without producing conidia or protoperithecia (Berlin, V. and C. Yanofsky 1985. Mol. Cell. Biol. 5:849-855). It is well known that some liquid media support production of macroconidia in static cultures (Weiss, B. and G. Turian 1966. J. Gen. Microbiol. 44:407-418). Here we report the occurrence of conidial differentiation in constantly agitated liquid cultures of Neurospora crassa upon starvation for nitrogen or limitation for glucose.

For our experiments we used Neurospora crassa wild type strain STa. A modified Vogel's medium (Russo, V.E.A. 1988. J. Photochem. Photobiol. 2:59-65) with 2% glucose instead of sucrose was employed as a standard growth medium; the pH was 6.8. Ammonium nitrate was replaced by NH4Cl or NaNO3 to provide only the reduced or oxidized form of nitrogen. 250 ml Erlenmeyer flasks containing 75 ml liquid medium were inoculated with 2 x 10^5 conidia/ml and grown in the dark in an incubator shaker (New Brunswick Scientific) at 150 rpm, 34°c. In a first series of experiments we grew the fungus for 24 h in a set of media differing in the amount and type of nitrogen sources and differing in glucose concentrations. Using the conditions listed in Table 1, we obtained different growth behavior of hyphae and conidiation or no conidiation at all. The cultures with conidia were orange, those without were white. Hyphae not only differ in the absence or presence of the conidiophores, but also in their overall macroscopic and microscopic structures. For instance, in medium containing 50 mM NH4Cl and 2% glucose, the fungus forms more or less separate hyphae with only short branches coming out from long hyphae, thus producing a homogenous distribution of the mycelium in the medium. On the other hand, the replacement of NH4Cl with NaNO3 (50 mM) causes the fungus to grow as a uniform big agglomerate. Hyphae of the agglomerate appear to have intertwined branches which produce a three-dimensional net-like structure. From the data presented in Table 1 we conclude that conidiation is induced within 24 h if the nitrogen is supplied at low concentration (2 fl). Another way to induce conidiation is to limit glucose in the presence of NH4Cl. Under these conditions, there appears to be no correlation between conidiation and pH of the medium or dry weights of the cultures.

Table 1. Effect of nitrogen source and glucose concentration on growth behavior, color and conidiation of hyphae (average of 2 experiments each with four replicates).

	Nitrogen Source	Glucose	Growth Behavior	Color	Conidia	mg dry weight per 75 ml culture	pH** after 24 h
1	50 mM NH4Cl	2%	S	w	no	300 ± 15	3.2
2	2 mM NH4Cl	2%	A	do	yes	76 ± 2	5.7
3	50 mM NH4Cl	2%	A	w	no	190 ± 25	6.3
4	2 mM NaNO3	**2%**	A,AS*	po	yes	75 ± 3	**6.2**
5	50 mM NH4Cl	**0.2%**	MSA	po	yes	110 ± 5	**5.4**
6	50 mM NaNO3	**0.2%**	AS	w	no	75 ± 3	**6.5**

A = one big agglomerate
S = no agglomeration, separate growth of hyphae
AS = between A and S
MSA= many small agglomerates

w = white
po = pale orange
do = deep orange

* Sometimes A, sometimes AS
** standard error of the mean was ± 0.1 pH

To test how fast the conidia are produced under agitation conditions, we grew hyphae in standard medium with 50 mM NH4Cl for 16 h. After that time, cultures had reached approximately the mid exponential growth phase. Cultures were carefully harvested onto sterile, 7 cm Schleicher and Schüll filter paper disks with a Büchner funnel under red safety light, washed twice with sterile, prewarmed (34°C) double distilled water and

resuspended in fresh standard medium without any nitrogen source. Hyphae were never allowed to dry during filtration. After transfer, the hyphae formed a very loose clump, probably as a result of the filtration procedure. Cultures were incubated further and examined for the presence of conidia at different times after transfer. Pieces of the mycelium were taken from the edge of the agglomerate and the percentage of hyphae that were septated into conidia was estimated using a light microscope. Pictures were taken from the hyphae (Figure 1). As controls we transferred hyphae to medium containing either NH4Cl or NaNO3, or NaCl (each 50 mM) and analysed the cultures after 12 h. The results are presented in Table 2. Fully septated conidia could be observed from 4 h after transfer (Figure 1b). Hyphae had white color at this stage but became pigmented during prolonged starvation, as the formation of conidia progressed. After 12 h incubation, the mycelial edge (about 3-5 mm) appeared very orange. In the control media with NH4Cl or NaN03 the mycelia lacked conidia and pigment. The addition of NaCl had the same effect as nitrogen starvation, an indication that the observed inhibition of conidiation by NH4Cl and NaN03 was not due to high concentration of Na^+ or Cl^-. It should be noted that under constant agitation in liquid medium, the formed conidia do not entirely separate from each other, but produce chains and arbusculete structures. The reason for this is unknown.

Table 2. Kinetics of condia production upon nitrogen starvation. Hyphae grown 16 h in reduction standard medium with 50 mM NH_4Cl were transferred to standard medium without nitrogen. Estimation of the percent hyphae septated into conidia was done on two pieces obtained from the edge of the agglomerate (average of two experiments each with two replicates).

hours after transfer to nitrogen-free medium	addition	% of hyphae septated into conidia*	pH	mg dry weight per 75 ml culture**
0	-	0	4.5	103
2	-	0	5.8	136
4	-	20	5.6	156
6	-	50	5.6	183
8	-	80	5.6	196
12	-	90	5.6	210
12	50 mM NaCl	90	5.5	213
12	50 mM NH_4Cl	0	3.9	312
12	50 mM $NaNO_3$	0	6.9	298

* standard error of the mean was about 20%
** standard error of the mean was about 5%

Our experiments indicate that starvation for nitrogen or limitation for glucose induces formation of conidia in the ascomycete Neurospora crassa. Using similar stepdown experiments it was already shown that nitrogen regulates another morphogenetic process, the formation of protoperithecia (Sommer et al. 1987. Planta 170:205-208). So, nitrogen starvation plays a key role in triggering both the asexual and sexual life cycles of Neurospora.

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