The Effects of Nitrogen Source Upon Respiration of Neurospora crassa

B.L. Sauer, H.J. Colvin and K.D. Munkres

Laboratories of Molecular Biology and Genetics

University of Wisconsin

Madison, Wisconsin 53706

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Summary:

Neurospora crassa exhibits two forms of respiration: 1) cyanide-sensitive; and 2) cyanide-resistant, salicyl hydroxamate-sensitive. The respiration of wild-type conidia can be made to resemble that of the conidia of extrachromosomal mutants by the appropriate selection of the preculture medium. This effect is not permanent, however; the normal wild-type respiratory pattern is re-established in growing mycelia. Moreover, the respiration of stationary phase wild-type mycelia can be made to mimic that of extrachromosomal mutant conidia. The nitrogen source and concentration appear to regulate the amount and form of respiration in wild-type. The mutants appear to be altered in such regulation.

Introduction

Mitochondrial respiration of wild-type N. crassa can be inhibited by cyanide (1). There are extrachromosomal mutants with abnormal mitochondrial respiration; that is, insensitive to cyanide. Lambowitz and Slayman (1) found this abnormal respiration is sensitive to salicyl hydroxamate (SHAM), that it occurs in wild-type in small amounts, and that its activity may be increased by growing wild-type in the presence of antimycin A, cyanide or chloramphenicol

The effects of nitrogen source and concentration upon the amount of cyanide-sensitive and cyanide-resistant, SHAM-sensitive respiration in conidia of wild-type and extrachromosomal mutants are reported. The respiration of wild-type N. crassa during growth and the effect of nitrogen source and concentration upon respiration are also described.

Materials and Methods

Strains. The wild-type was 74A-8. The extrachromosomal mutants were: [mi-1]f^- (RL 3367-21a); [mi-1]f^+ (RL 3603-1-1a); [mi-3] (RL 3593-25A); and [SG-1] (RL 3338-3A). f^+ is a nuclear gene that suppresses the lag period of growth of [mi-1] on solid media.

Media. The media were YEGCE (2), Wainwright's (3), Fries' (4), and modified Fries'. Modified Fries' and Fries' contain equal amounts of nitrogen. Nitrate is the sole source of nitrogen in modified Fries'.

Growth. Procedures were as described by Colvin and Munkres (5).

Respiration. Respiration was measured with a Gilson model KM-C polarograph. Specific rates of respiration are expressed as Q_{O_2} ($\mu l\ O_2\ mg^{-1}$ dry weight hr^{-1}).

Inhibition of respiration. After the initial rate of uninhibited respiration was determined, a stock KCN solution was added to the polarographic chamber to a final concentration of 1 mM, and the rate of cyanide-resistant respiration was measured.

After this measurement, the assay mixture was aerated to saturation and a stock solution of SHAM was added to the assay mixture to a final concentration of 0.68 mM.

Results

Effect of Preculture Medium Upon Conidial Respiration. Wild-type conidia were obtained from three types of media at daily intervals from 7- to 12-day-old cultures and the specific rates of respiration were measured (Table I). The proportions of the two forms of respiration were the same for conidia from YEGCE and Wainwright's media: the rates of cyanide-sensitive respiration were high and the rates of cyanide-resistant respiration were very low or zero. In contrast, conidia from Fries' exhibited low cyanide-sensitive and high cyanide-resistant respiration.

The only nutritional difference among these three media which correlates with the difference in respiratory characteris-

TABLE I

RESPIRATION OF CONIDIA OF WILD-TYPE N. CRASSA AFTER CULTURE AND CONIDIATION ON VARIOUS MEDIA

Form of Respiration	Specific Rate of Respiration[c] (Q_{O_2}, $\mu l\ O_2\ hr^{-1} mg^{-1}$ dry weight)						
	Culture Medium						
	Fries'		Modified Fries'	Wainwright's		YEGCE	
	Assay Medium						
	F	W	F	F	W	F	W
Terminal[a]	4±7	0±3	44	39±5	24±4	30±5	26±4
Alternate[b]	30±5	19±2	1	2.0 ±0.2	2.0 ±1.7	5.8 ±3.3	1.8 ±2.2

[a] cyanide sensitive

[b] cyanide resistant, salicyl hydroxamate sensitive

[c] Conidia were obtained from slants of the various culture media at daily intervals from 7- to 12-day-old cultures grown at 30°. They were washed from the slants with the assay medium. Aliquots of the suspension were used for measurement of cell dry weight and respiration. All media (except Water (W)) contained 2% glucose. After determining the uninhibited respiratory rate, terminal respiration was inhibited with 1.0 mM cyanide. Then the residual respiration was inhibited by titration with salicyl hydroxamate. The Q_{O_2} of these two forms of respiration did not vary more than 10% among 7- to 12 day-old conidia; therefore, the measurements from each of the 6 days was averaged.

tics is the form and quantity of the nitrogen source. Only Fries' is high in nitrogen concentration, primarily as ammonia. Conidia from a modified Fries' medium containing the same amount of nitrogen as nitrate with no ammonia exhibited respiration like conidia from either YEGCE or Wainwright's (Table I).

Conidial respiration of extrachromosomal mutants is summarized in Table II. Conidia of [mi-1], either with or without the suppressor gene f, and [mi-3] exhibited little or no cyanide-sensitive respiration and high cyanide-resistant respi-

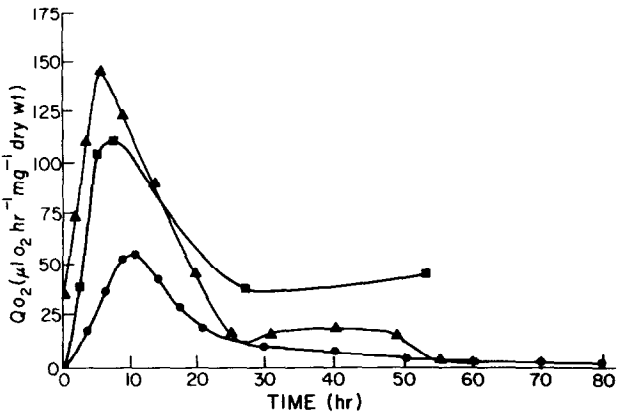

Figure 1. Specific cyanide-sensitive respiration of wild-type N. crassa as a function of the culture age and the type of culture medium.

Three culture media were used (Table I): Y (low ammonia, YEGCE); F (high ammonia, Fries'); and MF (nitrate, modified Fries'). Conidial inocula were obtained from slants of either Y or F and the incubations were in liquid medium of either F or MF. ▲ - ▲, Y → F; ● - ●, F → F; and ▨ - ▨, F → MF. Incubations were in shake cultures with 2% glucose at 30°.

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ration. Cyanide-sensitive respiration of [SG-1] conidia was equivalent to wild-type and the cyanide-resistant respiration was greater than wild-type. The respiration of [mi-3] from Fries' did not differ from that of [mi-3] from YEGCE.

The Effect of Preculture Medium Upon Respiration of Wild-Type Mycelia. The growth rates in Fries' medium were not affected by the type of preculture medium. There were significant differences, however, in the two forms of respiration (Figures 1 and 2). Cultures with inocula from YEGCE exhibited a rapid increase in the specific rate of cyanide-sensitive respiration and little cyanide-resistant respiration. However, cultures with inocula from Fries' developed cyanide-sensitive respiration at a slower rate and the cyanide-resistant respiration increased to a maximum at three hours and then declined to zero. In stationary phase mycelia, there was little cyanide-

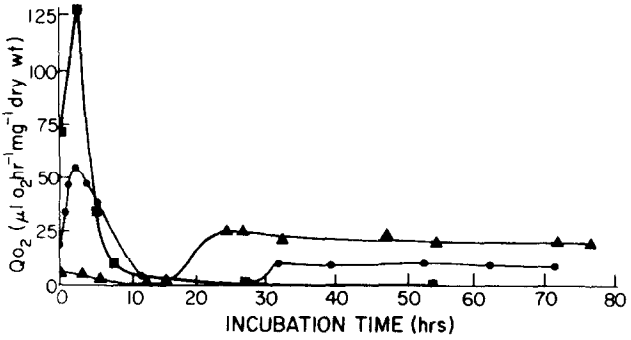

Figure 2. Specific cyanide-resistant respiration of wild-type N. crassa as a function of the culture age and the type of culture medium.

Conditions and symbols as in Figure 1.

sensitive respiration and relatively high cyanide-resistant respiration regardless of the type of preculture medium. The activities of both forms of respiration in stationary phase mycelia on Fries' were comparable to those of conidia from Fries'.

When the modified Fries' medium was inoculated with conidia from Fries', cyanide-resistant respiration initially followed the same pattern as with conidia from Fries' into Fries' (Figure 1). In stationary phase, however, cyanide-sensitive respiration was high and cyanide-resistant respiration was low or absent. Thus the forms and rates of respiration of stationary phase mycelia after culture on modified Fries' are similar to those of conidia from either modified Fries', YEGCE or Wainwright's.

Discussion

The development of the two types of respiratory systems in wild-type Neurospora appears to be subject to both genetic and regulatory controls. The form and concentration of the nitrogen source in the medium and the oxygen tension (6) are regulatory

Table II
RESPIRATORY ACTIVITIES OF CONIDIA OF WILD-TYPE AND EXTRACHROMOSOMAL MUTANTS OF *N. CRASSA*

Strain	Preculture Medium[a]	Specific Rate of Respiration[b] (Q_{O_2}, µl O_2 hr^{-1} mg^{-1} dry weight)	
		Form of Respiration	
		Terminal	Alternate
wild-type (74A8)	Y	30±5	6±3
	F	4±7	30±5
mutant:			
[mi-1]f$^-$	Y	0±3	25±3
[mi-1]f$^+$	Y	0±3	40±7
[mi-3]	Y	1	22
	F	0.4	38
[SG-1]	Y	32±6	12±2

[a] Y, YEGCE; F, Fries'

[b] Conidia were obtained from 7- to 14-day-old cultures. Assays were with Fries' containing 2% glucose. Other procedures as in Table I.

factors. These results and additional (6) studies indicate that regulatory controls are altered in the extrachromosomal mitochondrial mutants of *N. crassa*. The genetics and regulation of respiration and fermentation will be discussed elsewhere (6) in terms of the dynamic repressor control model (7).

The mechanism(s) by which ammonia and nitrate serve in the regulation of the development of the mitochondrial respiratory systems remain to be determined. Hypothetically, these ions may act as co- or anti-repressors (6). Perhaps the regulation of the synthesis and catabolism of nitrate reductase by ammonia and

nitrate in Neurospora (8,9) may prove to be analogous to the regulation of the respiratory system.

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