

## Modification by light of nitrite release and accumulation by *Neurospora crassa*

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Activity of nitrate reductase (NR) induced by nitrate in the growth medium can be tested by diazotation of the nitrite formed during an activity test (Snell and Snell, 1949, Colorimetric Methods of Analysis, pp. 802-807). Pink instead of colorless controls (NADPH omitted) in these tests indicated nitrite present in the samples without NR activity and caused us to assay for nitrite excreted into the culture medium and for nitrite accumulated in the mycelia of *Neurospora crassa* strain *al-2;bd*.

Conidia for inoculation of the experimental cultures were washed from conidiating mycelia in darkness under sterile conditions and kept in sterile water in darkness at 5°C. For the experiments they were used between 1 and 14 days after harvest. *Neurospora* mycelia were grown from these conidia (1.5 x 10<sup>8</sup>) per 300 ml in 2 l Erlenmeyer flasks) with modified Vogel's medium (25 mM NH<sub>2</sub>NO<sub>3</sub> replaced by 50 mM NaNO<sub>3</sub> and supplemented with 1% glucose) on a gyratory shaker at 80 rpm and 30°C for 16 h. In each experiment four independent culture flasks were kept in darkness, and four received 3W m<sup>-2</sup> of white light during their growth phase. The eight mycelia were harvested separately by filtration and the eight filtrates (each 300 ml medium) kept for nitrite assays. Three hundred milligrams of each washed mycelium was permeated for 30 min with a permeation solution containing 5% propanol (W. Ullrich, pers. commun.) and assayed for accumulated nitrite. NR activity was determined according to established procedures (Garrett and Nason 1969. J. Biol. Chem. 244:2870-2882) modified by Scheideler and Ninnemann 1986. Analyt. Biochem. 154:29-33.

Large amounts of nitrite were produced by mycelia grown on nitrate as sole nitrogen source: Table 1 shows the amounts of nitrite released into the medium or accumulated in the mycelia after 16 h of growth in NO<sub>3</sub><sup>-</sup> medium and kept in darkness (D) or in light (L). The values were standardized by relating them to 1 g mycelium (fresh weight).

**Table 1:** Release and accumulation of nitrite by *Neurospora crassa al-2;bd* grown for 16 h in NO<sub>3</sub><sup>-</sup> medium.

| Age of<br>conidia for<br>inoculation<br>of cultures | FILTRATE<br>μ moles NO <sub>2</sub> <sup>-</sup> |            | MYCELIUM<br>μ moles NO <sub>2</sub> <sup>-</sup> |            | TOTAL NO <sub>2</sub> <sup>-</sup><br>μ moles |      |
|-----------------------------------------------------|--------------------------------------------------|------------|--------------------------------------------------|------------|-----------------------------------------------|------|
|                                                     | g fr. wt.                                        |            | g fr. wt.                                        |            | g fr. wt.                                     |      |
|                                                     | L                                                | D          | L                                                | D          | L                                             | D    |
| 2 days                                              | 27.2 ± 1.9*                                      | 40.7 ± 5.6 | 19.9 ± 1.5                                       | 18.9 ± 2.5 | 47.1                                          | 59.6 |
| 5 days                                              | 39.7 ± 0.7                                       | 47.9 ± 4.2 | 22.7 ± 0.9                                       | 19.5 ± 1.6 | 62.4                                          | 67.4 |
| 7 days                                              | 19.5 ± 5.8                                       | 35.1 ± 3.5 | 24.5 ± 1.2                                       | 17.6 ± 3.5 | 44.0                                          | 52.8 |
| 10 days                                             | 19.0 ± 2.4                                       | 24.3 ± 3.6 | 19.6 ± 1.5                                       | 16.8 ± 3.7 | 38.6                                          | 41.1 |

\* standard deviation

The absolute amounts of nitrite released and accumulated varied between the standardized experiments on various days. Our suspicion that the age of the aqueous conidial stock solution used for inoculation of the experiments might influence the extent of nitrite formation of the mycelia was not substantiated. But independent of the absolute amounts of nitrite, significantly more nitrite was always found in filtrates of dark grown mycelia than in those of irradiated ones. The amounts of nitrite accumulated in mycelia were about equal in dark and light grown *Neurospora* with a tendency of homogenates of each culture and showed no difference between dark and irradiated mycelia (values of 500-1000 nmoles  $\text{NO}_2^-$  formed/mg protein in 10 min at 30°C).

The added amounts of accumulated plus excreted nitrite ("total  $\text{NO}_2^-$ ") of dark mycelia exceeded total nitrite of irradiated cultures (Table 1, column 3), i.e. in all, dark mycelia reduced less nitrite to ammonia than irradiated ones.

The high amounts of nitrite found in the media and especially in mycelia suggest that the rate of nitrate reduction exceeds the rate of nitrite reduction. The amounts of nitrite excreted into the medium resulted in  $\text{NO}_2^-$  concentrations of 150-250  $\mu\text{M}$  for cultures grown for 16 h in nitrate medium. The same order of magnitude of released nitrite was reported for green algae under low  $\text{CO}_2$  tension and high irradiance (Azucara and Aparicio 1983. *Plant Physiol.* 71:286-290; Quinones and Aparicio, in: *Inorganic Nitrogen Metabolism*, W.R. Ullrich et al., eds. in press). A rough estimate of the minimal nitrite concentration in the mycelium can be based on 1 g fresh mycelium corresponding to 1.0-1.5 ml volume. If one third of it (or less) is cytoplasmic space, the detected amounts of 10-20  $\mu\text{moles}$  nitrite per g mycelium (fresh weight) equal 20-60 mM nitrite in *Neurospora* cells, a concentration the cells should have to compartmentize in order not to become poisoned (with 16 mM nitrite as nitrogen source, *Neurospora* does not grow anymore, Ninnemann, unpubl.).

The data of Table 1 conform to the hypothesis that nitrite reductase of *Neurospora* becomes activated by irradiation applied during culture growth resulting in lower amounts of total nitrite (accumulated plus released). A similar conclusion was reached for the system of *Monoraphidium braunii*, in which blue light appears to be required for the biosynthesis of nitrite reductase (Quinones and Aparicio, in: *Inorganic Nitrogen Metabolism*, W.R. Ullrich et al., eds. in press). Also, a change of nitrate uptake and nitrite export systems by light might be considered.

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