Effect of light on perithecial production in homothallic Neurospora species

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Abstract
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tetrahexosylceramide have been previously reported by Lester et al. (1947, J. Biol. Chem. 249: 3388). Structure of this compound was established only on basis of GLC or TLC chromatographic mobilities. Other evidences (Kushwaha, et al., 1976, Lipids, 11: 778) denied the existence of glycolipids in Neurospora mycelium. - - - - Instituto de Investigaciones Biológicas “Fundacion Campomar” and Departamento de Química Organica, Facultad de Ciencias Exactas y Naturales. Obigado 2490, 1428 Buenos Aires, Argentina.

Nenê, L. and H. Ninnemann

Continuous irradiation of young (2-6 h) liquid cultures of Neurospora crassa wild type 74-CR23-1A (FGSC #987) can induce cyanide-insensitive respiration (for review of such respiration see Solomons 1977 Ann. Rev. Plant Physiol. 28: 279). Rather high intensities of white light were needed to induce (>450 W/m²) measured in front of the culture vessel, about 45 W/m² behind it; light sources: Atralux 23OV, 300 W Osram or mercury high pressure lamps HQA 125 W Osram) which also decreased the growth rate. Neurospora was grown at 25-30°C in 2 l-Erlenmeyer flasks with 300 ml Vogel’s minimal medium containing 2% sucrose (Vogel 1956 Microbiol. Genet. Bull 13: 42) on a reciprocal shaker with 140 strokes per min. Media were inoculated with 10⁸ conidia/ 300 ml: Irradiation started 2 h after inoculation. A slow increase of temperature in the cultures (maximally 5-12°C) could not be prevented with the intercalated heat absorbing filters (K₁, Schott), but growing parallel wrapped cultures or cultures at 37°C in darkness could not induce cyanide-insensitive respiration.

Respiratory rates via the cyanide-insensitive respiration pathway were determined in the presence of 2 mM KCN with an oxygen electrode, via the cyanide-sensitive way in the presence of 1.5 mM salicyl hydroxamic acid (SHAM). These values do not add up to 100% since electron transport is not fully operative in both pathways before addition of the respective inhibitor. Cyanide insensitivity did not appear immediately with the onset of irradiation but showed a lag phase of 2-4 h. The cyanide-insensitive respiration decreased after 6-8 h of irradiation or after transferring the cultures into darkness. Irradiation of older cultures did not induce cyanide-insensitive respiration.

Cyanide-insensitive respiration of a mycelium irradiated for 8 h with 450 W/m² was 42% of the uninhibited respiration (i.e. no inhibitor added); cyanide-sensitive respiration was 73% of the total. The dark control was ≥96% cyanide-sensitive.

In isolated mitochondria from irradiated mycelium the cyanide-insensitive and SHAM-sensitive respiration with succinate was 30% of the uninhibited respiration, and with exogenous NADH it was 38%.

Since NADH dehydrogenase, succinate dehydrogenase and ubiquinone are involved in both pathways, we do not believe that cyanide-insensitive respiration is induced by their photoinactivation (see Ramadan-Talib and Prebble 1978 Biochem. J. 176: 767) under our conditions. (Supported by the Deutsche Forschungsgemeinschaft.) - - - - Institut fur Chemische Pflanzenphysiologie, Corrensstr. 41, 74 Tubingen, West Germany.

Raju, N. B.

Evidence suggesting that light may be required for perithecial production in homothallic species of Neurospora came two years ago from experiments of Stuart Brady on ascospore shooting. Subsequent determination of five homothallic Neurospora species show marked differences in their abilities to fruit under different light conditions. All five species fruit well on minimal medium at 25°C using an alternating light-dark regimen.

Perithecial production of N. africana (FGSC #1740), N. dodgei (FGSC #1692), N. galapagosensis (FGSC #2290) and N. lineolata (FGSC #1910) is drastically reduced either by continuous fluorescent light (about 1000 lux units) or by continuous dark. In contrast, all four species form a similar number of perithecia in an alternating 12 h light-dark regimen or in diurnal conditions. Cultures of the four homothallic species that produce few or no perithecia in continuous light (or dark) subsequently form numerous perithecia after exposure to an alternating light-dark regimen.

Another homothallic species, N. terricola (FGSC #1899), is, however, unaffected by light/dark conditions. Similarly, the pseudohomothallic, N. tetrasperma (FGSC #1270 and 1271), with both A and a strains inoculated simultaneously is also unaffected by light or dark. Light has no effect on fruiting of the homothallic Sordaria macrospora (Esser 1980, Mycologia 72: 619). However, in Gelasinospora reticulospora, exposures to both light and dark are necessary to induce perithecial formation (Inoue and Furuya 1970, Development, Growth and Differentiation 12: 141; additional references may be found in "The Filamentous Fungi", Vol. 3, Chapters 16 (G. Turian) and 17 (K. Tan), eds J.E. Smith and D.R. Berry, 1978. John Wiley & Sons, New York). These preliminary observations indicate that both light and dark influence perithecial...
formation in some species, but not in others. Since perithecial production is significant for laboratory genetics as well as for the photobiology and ecology of Neurospora, observations should be extended to the four known heterothallic species. (Supported by Public Health Service Research Grant AI 01462.)

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Department of Biological Sciences, Stanford University, Stanford, California 94305.

Selitrennikoff, C. P.

A new allele of csp-2 which does not complement csp-1.

The conidial separation mutations [csp-1 (IL) and csp-2 (VII)] confer upon Neurospora strains reduced ability of conidial chains to separate; double mutant strains are even further impaired (see Selitrennikoff et al. 1974 Genetics 78: 679). The one csp-2 allele and the three csp-2 alleles complement in heterokaryons and form a wild type number of free conidia. A new allele of csp-2 is described which does not complement csp-2 yet is recessive to wild type.

A strain of poky f which did not form free conidia was obtained from John Chalmers (University of California, Berkeley) and was found to contain a conidial separation mutant. This mutant was crossed to wild type (Oak Ridge) four times (as the male parent) and the csp phenotype segregated 1:1. This mutant strain, designated UCLA 102, was found to grow in both liquid stationary culture and "race" tubes identically with wild type. Inter se crosses among the csp strains showed that UCLA 102 was allelic with csp-2 (0 recombinants/69 with FS 590; 0/211 with FS 591; 9176 with UCLA 101) but was not allelic with csp-1 (1/40). The double mutant, csp-1;csp-2 (UCLA 102) was found to produce a very low level of free conidia, similar to other combinations of csp-1 and csp-2 alleles. The phenotype of forced heterokaryons containing csp-1 and csp-2 alleles is essentially wild type as judged by both free conidia with a haemocytometer or inverting agar-slant cultures and tapping to release free conidia (the "tap-test"; Selitrennikoff and Nelson 1973 Neurospora Newsl 20: 34). In sharp contrast, the forced heterokaryon csp-1/csp-2 (UCLA 102) produced a number of free conidia equivalent to the csp-2 level, i.e., csp-2 (UCLA 102) did not complement with csp-1. However, csp-2 (UCLA 102) was found to be recessive to csp-2+.

These results indicate that the interaction of the csp genes (or gene products) is not as autonomous as previously described. The new allele of csp-2 is available from the Fungal Genetics Stock Center.

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Smith, B. R. and M. E. Yorston

Crossoes between the complementing histidine-5 allele K78 and K746 yield two kinds of his+ progeny. One results from recombination between the alleles and the other primarily from nondisjunction of chromosome IV giving disomic ascospores. Less frequently, disomic progeny may be produced as a result of extra chromosome replication or chromosome non-conjunction (pairing failure). The disomic spores form pseudowild colonies on minimal medium that are distinguishable from the much rarer his+ recombinants by their slower growth rate.

The inclusion in the parent strains of linked auxotrophic markers, flanking the his-5 locus, permits easy recognition of parental and pseudowild types among conidia formed by pseudowilds. In contrast, conidia from his+ recombinants are homokaryotic and do not show marker segregations.

Genotypes of parent strains.

<table>
<thead>
<tr>
<th>Parent A</th>
<th>Parent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyr-3 (1298), his-5 (K78), a</td>
<td>his-5 (K746), leu-2 (37501), A</td>
</tr>
</tbody>
</table>

Reciprocal crosses were prepared, some of which were treated with p-fluorophenylalanine. The progeny were then screened on selective media to estimate the frequency of pseudowilds. The amino acid analogue p-fluorophenylalanine is known to increase meiotic non-disjunction of chromosome I of Neurospora (Griffiths and DeLange. Mutat. Res. 1977, 46: 345) and should significantly increase frequencies of pseudowilds in these crosses.

Preparation and treatment of crosses

Petri dishes (90 mm diameter) containing 20 ml of Westergaard's crossing medium supplemented with uracil, histidine and leucine, were inoculated with drops of conidial suspension of one parent. To increase the fertility of the crosses, macerated Whatman's No. 1 filter paper was added to the crossing medium at the rate of 270 cm²/1. Petri plates were incubated for five days to allow protoperithecia to form. During this period, the petri dish walls were wiped with alcohol twice daily to prevent the mycelium spreading over the sides of the plates. Fertilization was then effected by the addition of a dense suspension of conidia. Excess water was removed after 30 min and 4½ h later, 5 ml of water or 5 ml of p-fluorophenylalanine solution (0.05 mg/ml) was added to each petri plate. The water or p-fluorophenylalanine solution