Ubiquinone in mitochondria of cytoplasmic mutants

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Abstract
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Ubiquinone content of mitochondria of respiratory cytoplasmic mutants of N. crassa.

We examined, in our experiments, the following strains: wild type 74-OR23-1A (FGSC#987), [mi-4] A (FGSC#1583), and [mi-1] nio-2 A (FGSC#1576). Test tubes containing 10 ml of Vogel's minimal medium supplemented with 1% sucrose and 1.5% agar were inoculated and cultured at 34°C for 5 days. Conidia from one test tube were harvested in 10 ml of water and transferred to 2-liter Erlenmeyer flasks containing 1 liter of Vogel's minimal medium supplemented with 1% sucrose and incubated in a shaker at 34°C for 46 hours.

For the isolation of mitochondria, mycelia were collected on a double layer of cheesecloth, washed with distilled water and suspended in the medium of Munkres et al. (1966 Neurospora News l. 9: 14). 5 ml of medium per 1 g of moist weight of mycelium. The suspension was ground with powdered glass in a cold mortar for a minute and squeezed out through a double layer of cheesecloth. The filtrate was centrifuged at 1,000 x g for 10 minutes. The pellet was discarded and the supernatant was further centrifuged at 20,000 x g for 30 minutes. The crude mitochondrial fraction obtained was washed once with the isolation medium, centrifuged once more at the same force and purified by the double-shelf technique according to Munkres et al. (loc. cit.). Mitochondria were collected from the interphase, washed twice with 0.25 M sucrose, 0.05 M tris-HCl, 0.007 M EDTA pH 7.4 and suspended in the same medium to contain about 25 mg of protein per 1 ml.

Mitochondrial protein was determined by the biuret method according to Szarkowska and Klingenberg (1963 aichem. Z. 338:674). Ubiquinone was determined essentially according to the procedure of Pumphrey and Redfern (1961 Biochem. J. 76:61), with the modification described previously by Drabikowska and Szarkowska (1965 Acta aichem. Polon. 12:387).

We have found that the two cytoplasmic mutants analysed contained 5-6 μmoles of ubiquinone per 1 g of protein, which is almost 3 times that found in the wild type of N. crassa (ca. 1.5 μmoles/g protein). It was established also that ubiquinone takes part in the oxidoreductive processes. The results of a more extensive study of the ubiquinone function will be published elsewhere.

I should like to thank D. D. Perkins for suggesting the use of AR190, for breeding MN58p into this translocation and for making his unpublished observations available to me. — *Research School of Biological Sciences, Australian National University, Box 475 GPO, Canberra, A.C. T., Australia.*