Fungal Genetics Reports

Volume 15 Article 3

Ubiquinone in mitochondria of cytoplasmic mutants

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Abstract

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

The [mi-1], also called poky, and [mi-4] mutants are known as respiratory cytoplasmic mutants which differ from the wild type in their content of cytochromes. Both of these mutants, when analyzed recently in this laboratory, also show great differences in ubiquinone content, as compared with the wild type.

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 Newsl. 9: 14), 5 ml ofmedium per 1 g of moist weight of mycelium. The suspension was ground with powdered glass in a cold mortar for a minutes and squeezed out through a double layer of cheese cloth. 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, 0.001 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 aiochem. Z. 338:674). Ubiquinone was determined essentially according to the procedure of Pumphrey and Redfearn (1960 Biochem. J. 76:61), with the modification described previously by Drabikowska and Szarkowska (1965 Acta aiochim. Polon. 12:387).

We have found that the two cytoplasmic mutants analysed contain 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.a 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. • • • Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa 12, Rakowiecka 36. Poland.