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SHORT COMMUNICATION

PRESENCE OF β-CAROTENE IN CULTURES OF *MORTIERELLA RAMANNIANA* VAR. *RAMANNIANA*

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Abstract—β-Carotene has been found as the only pigment in cultures of *Mortierella ramanniana* var. *ramanniana*.

INTRODUCTION

ALTHOUGH it is a characteristic of many members of the order Mucorales to accumulate carotenoids both in vegetative hyphae and sporangiophores,[1,2] it has been reported that a group of mucoraceous fungi, including *Mucor ramannianus* (*Mortierella ramanniana*),[3] do not accumulate carotenoid pigments to any detectable degree. In this laboratory cultures of *Mortierella ramannaina* var. *ramanniana*[4] have been shown to accumulate large amounts of pigment in the mycelium.

RESULTS

The pigment extract was chromatographed on columns of $Ca(OH)_2$ and MgO–Supercel (1:1, w/w) and the single yellow pigment obtained did not fluoresce in u.v. light and was homogenous by TLC. It was identified as β-carotene by the following criteria: (i) when partitioned between equal volumes of petroleum ether, b.p. 40–60°, and methanol (90% and 95%) the pigment remained in the epiphase; (ii) its spectrum was identical with that of authentic β-carotene with max in petroleum ether, b.p. 40–60° (425, 451, 478 nm), in benzene (435, 462, 487 nm) and in CS_2 (450, 495, 520 nm); (iii) co-chromatography of the pigment with authentic β-carotene on TLC in three solvents; (iv) when dissolved in $CHCl_3$ and mixed with $SbCl_3$ in $CHCl_3$ (Carr–Price Reagent) a dark-blue coloration was produced which had an absorption maxima at 590 nm characteristic of β-carotene.

EXPERIMENTAL

Culture Conditions and Extraction Procedure

The strain of *Mortierella* used was obtained from the Botany Department, University of Nottingham. Stock cultures were maintained on Czapek Dox potato dextrose agar. Erlenmeyer flasks (100 ml) containing a basal medium (20 ml) of glucose 5%, KH_2PO_4 0·1%, $MgSO_4$ 0·05% in citrate buffer 0·2 M, pH 4·5, supplemented with urea 2·8%, thiamine 0·005 M and trace elements,[5] were inoculated with a spore suspension

[1] T. W. GOODWIN, *Biochem. J.* **50**, 550 (1952).
[2] D. M. THOMAS and T. W. GOODWIN, *Phytochem.* **6**, 355 (1967).
[3] D. HOCKING, *Nature* **197**, 404 (1963).
[4] M. TURNER, *Trans. Brit. Mycol. Soc.* **46**, 262 (1963).
[5] A. G. MORTON and A. MACMILLAN, *J. Exptl. Botany* **5**, 232 (1954).

(4×10^7 spores). The fungus cannot utilize urea or citrate as a sole source of carbon. After 7 days' incubation at $25° \pm 2°$ in the presence of light on a New Brunswick rotary-type shaker, the growth was harvested by filtration, the unsaponifiable fraction extracted by standard methods[6] and finally dissolved in redistilled petroleum ether, b.p. 40–60°.

Purification

Column chromatograms were developed on $Ca(OH)_2$ and MgO–Supercel (1:1, w/w) with petroleum ether, b.p. 40–60°, and hexane containing increasing amounts of acetone respectively. TLC was on silica gel using (i) petroleum ether, b.p. 40–60°, containing 5% Et_2O (v/v), (ii) benzene–isopropanol–acetone (100:8:1, v/v/v) and (iii) petroleum ether (b.p. 90–110°)–benzene (50:50, v/v).

[6] T. W. GOODWIN, *Modern Meth. Plant Anal.* **3**, 272 (1955).