References and Notes

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Increased Production of Carotene by Mixed + and – Cultures of Choanephora cucurbitarum

The production of carotenoid pigments by filamentous fungi is apparently quite common, but knowledge of their functions in fungus metabolism is meager. There is evidence that β-carotene is concerned with phototropic responses of certain fungi, and in many fungi, visible amounts of carotenoids are produced only in the reproductive structures. Goodwin (*1*) has reviewed the literature on this subject. *Phycomyces blakesleeanus* has been the principal test fungus, and the mycelium of either the + or the – sex (*2*) has been used. *Choanephora cucurbitarum* is not mentioned as a carotene producer.

Choanephora cucurbitarum is heterothallic (*3*) and zygospores have been observed and described (*4, 5*). No mention has been made of carotenoids in the mycelium, and only Wolf (*5*) reports observing "numerous yellowish oil globules" in immature zygospores.

During routine culturing of *C. cucurbitarum,* it was observed that the mycelium of combined + and – cultures in liquid medium became bright yellow within a few days, while the mycelium of either the + or – sex cultured alone was only slightly yellowish. This paper (*6*) reports results of subsequent experiments that show that the production of β-carotene by *C. cucurbitarum* is greatly increased in mixed + and – culture.

The + and – cultures of *C. cucurbitarum* used in this study were isolated from the same diseased pumpkin flower at Morgantown, W. Va., in 1954. A liquid medium (glucose, 25 g; acid-hydrolyzed casein, 2 g/lit, essential salts, and thiamine) at an initial *p*H of 6.0 was used.

The fungus was cultured at 25°C in 250-ml flasks containing 25 ml of medium without agitation and at 28°C in 9-lit bottles containing 6 lit of medium through which sterile air bubbled continuously. The experiments have been repeated a number of times with similar results, although quantitative carotene determinations were made on only some of the cultures.

Under both sets of cultural conditions, the mycelium in the mixed + and – cultures began to show yellow pigmentation after about 3 days, reaching a maximum intensity about the fifth or sixth day. The + and – mycelia produced little yellow pigment when they were grown separately.

Under all conditions of growth, the mycelium contained much oil after a few days, but only the mycelium in the mixed cultures showed conspicuous yellow pigments in the oil. Later, during sexual reproduction, these pigments usually became concentrated in the suspensor cells that subtend the immature zygospores. Some yellow pigment is evident in the oil droplets in immature zygospores.

The yellow mycelium was squeezed between layers of cloth to remove excess water, and drying was completed with absolute methanol. The pigments were extracted with petroleum ether, saponified and reextracted with petroleum ether. The β-carotene content of each extract was determined by measuring the optical density of 460 mμ in a Beckman photoelectric spectrophotometer according to the procedures of Garton *et al.* (*7*).

By using chromatographic adsorption techniques (*7*) and repeated recrystallizations from a 1-to-1 solution of ethanol and petroleum ether (bp 40° to 60°C), it was possible to isolate the primary pigment. This material was characterized by the use of chromatographic adsorption techniques (*7*), molecular extinction curves and extinction values ($E_{1\ cm}^{1\ percent} = 2400$ at 455 mμ in cyclohexane) (*8*), and identified as β-carotene. The yields of β-carotene in one typical experiment are presented in Table 1.

Under these conditions, the mycelium grown in mixed + and – cultures produced 15 to 20 times as much β-carotene per gram of mycelium as did that of either sex grown alone. The evidence suggested that the stimulatory substances responsible for the enhanced carotene production were secreted by the mycelium of the opposite sex.

When the + and – mycelia are grown on opposite sides of a cellophane membrane, both mycelia usually show increased pigment production, indicating that the stimulating substances are diffusible through cellophane and that the effect is reciprocal. The failure of these cultures to form zygospores after further incubation is evidence that the mycelium did not penetrate the cellophane.

Increased carotene production by the mycelium in mixed + and – cultures of a fungus does not seem to have been previously reported. Since there is normally no anastomosis between the + and – vegetative hyphae of the Mucorales, which would result in heterocaryotic mycelium, the stimulation in *C. cucurbitarum* must originate as secretions from the mycelium of the opposite sex. These secretions are possibly of the nature of hormones. It seems probable that some relationship exists between the production of carotene and sexual reproduction in *C. cucurbitarum.*

Table 1. Weight of dry mycelium and β-carotene after 6 days in 6-lit aerated cultures when + and – sexes were grown separately and together.

Culture	Dry mycelium (g)	β-carotene (μg)	Amt. β-carotene in dry mycelium (μg/g)
+	25	1,140	45.6
–	22	1,377	62.6
±	18	16,560	920.0

These investigations, as well as others designed to give more information on the role of carotenoids in the fungi and studies particularly concerning the relationship of carotene to sexual reproduction of *C. cucuribitarum,* are being continued.

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References and Notes

1. T. W. Goodwin, *Botan. Rev.* 18, 291 (1952); *Ann. Rev. Biochem.* 24, 497 (1955).
2. The term *sex* is used in this paper, following E. A. Bessey, *Morphology and Taxonomy of Fungi* (Blakiston, New York, 1950) and E. A. Gaumann, *The Fungi* (Hafner, New York, 1952), in preference to the less specific term *strain.*
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6. Published with the approval of the director of the West Virginia Agricultural Experiment Station as scientific paper No. 511.

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Fate of Radiostrontium Fed to Habrobracon Females

Strontium-89 has been of interest to us, not only because it is a biologically important product of nuclear fission, but more specifically because it is the only pure beta-emitting radioisotope with which we have been able to produce per-