glyoxals are present together, and therefore phenylglyoxal prevents the factor from accelerating the conversion of methylglyoxal to lactic acid as was shown by Hopkins & Morgan (1948).

4. The presence of uncombined methyl- or

phenylglyoxal accelerates the breakdown of the intermediate compounds.

The authors wish to thank Dr M. Dixon and Mr E. J. Morgan for details of the purification method for glyoxalase and for gifts of 'Yamazoye' intermediate compounds.

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Studies in Carotenogenesis

5. CAROTENE PRODUCTION BY VARIOUS MUTANTS OF *PHYCOMYCES BLAKESLEEANUS* AND BY *PHYCOMYCES NITENS*

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(Received 1 April 1952)

The general conditions governing carotene synthesis in *Phycomyces blakesleeanus* have been worked out in detail (Garton, Goodwin & Lijinsky, 1951) and the polyenes which accompany β -carotene (the major component) in traces have also been identified (Goodwin, 1952). As a number of morphological mutants of *P. blakesleeanus* have been isolated and were available, it was considered desirable to examine them quantitatively and qualitatively for carotene synthesis. It was thought possible that some metabolic block might have occurred which would help in the elucidation of the pathway of biosynthesis of carotene. The mutants examined were *P. blakesleeanus* var. *piloboloides* (+ and - strains), *P. blakesleeanus* mutant *gracilis* (+ strain only), *P. blakesleeanus* mutant *pallens* (- strain). *P. nitens* (+) was also examined.

A short account of this work has already appeared (Goodwin & Griffiths, 1952).

EXPERIMENTAL

Cultures. All the cultures used were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Cultural methods. The fungi were grown and analysed for dry weight, lipid and carotene production as described in detail by Garton *et al.* (1951). The standard medium containing 2.5% (w/v) glucose was used throughout.

Identification of the polyenes present. The methods described by Goodwin (1952) were used.

RESULTS

The polyenes produced. A chromatographic examination of the polyenes present in the various mutants was carried out. All the mutants contained the same polyenes as are found in the parent strain, namely: lycopene, neurosporene, α -, β -, γ - and δ -carotene, phytofluene and a phytoene-like substance. These were also present in essentially the same relative amounts as in the parent form of *P. blakesleeanus*.

Production of carotene. Typical results obtained with the various strains are recorded in Table 1 and Figs. 1 and 2. In the first place it will be seen from Fig. 1 that there are no significant differences between the three (-) strains in either the rate of synthesis or the amount of carotene finally produced. There is also no major difference between the amount of dry weight and lipid synthesized by these strains (Table 1).

When the (+) strains are considered (Fig. 2), *piloboloides* and *nitens* fall into line with the (+) strain of the parent in synthesizing about half as much carotene as the (-) strain (Garton *et al.* 1951), but the same amount of dry weight and lipid (Table 1). The mutant *gracilis* (+), however, is different in two respects: (a) it synthesizes as much carotene as the (-) strains of the other variants, and (b) the rate of synthesis in young cultures is very

Table 1. *The dry weight, lipid and carotene production by various strains of Phycomyces blakesleeanus and by Phycomyces nitens when grown on a standard medium containing 2.5% (w/v) glucose*

(The amounts recorded are those produced in an 8 oz. medicine bottle containing 15 ml. of medium.)

Days after inoculation ...	3				4				5			
Strain used	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)
<i>P. blakesleeanus</i> (-)	42	7.3	17.4	11	77	19.3	25.1	26	74	20.3	27.3	43
<i>P. blakesleeanus</i> var. <i>pilobolus</i> (+)	40	7.2	18.0	4	70	13	18.6	20	80	18.5	23.2	85
<i>P. blakesleeanus</i> var. <i>pilobolus</i> (-)	52	9.7	18.7	9	66	14.8	22.4	22	78	20.4	26.2	43
<i>P. blakesleeanus</i> mutant <i>gracilis</i> (+)	59	15.0	25.4	36	—	—	—	—	65	16.6	25.5	79
<i>P. blakesleeanus</i> mutant <i>pallens</i> (-)	36	6.9	19.1	9	66	8.4	12.7	16	79	12.9	16.3	44
<i>P. nitens</i> (+)	57	15.8	27.8	20	—	—	—	—	65	15.7	24.1	29

Days after inoculation ...	7				9				11			
Strain used	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)	Dry wt. (mg.)	Lipid (mg.)	Lipid (%)	Carotene (μg.)
<i>P. blakesleeanus</i> (-)	96	16.1	16.8	92	79	15.7	19.9	97	85	14.0	16.5	100
<i>P. blakesleeanus</i> var. <i>pilobolus</i> (+)	79	15.1	19.1	30	86	20.3	23.6	35	77	12.0	15.6	35
<i>P. blakesleeanus</i> var. <i>pilobolus</i> (-)	—	—	—	—	96	24.8	25.8	97	81	17.0	21.0	95
<i>P. blakesleeanus</i> mutant <i>gracilis</i> (+)	80	17.5	21.9	99	80	18.1	22.6	108	—	—	—	—
<i>P. blakesleeanus</i> mutant <i>pallens</i> (-)	102	23.7	23.2	115	97	28.2	10.9	109	—	—	—	—
<i>P. nitens</i> (+)	67	12.5	18.6	31	62	10.5	16.9	24	—	—	—	—

Fig. 1. The concentration of β -carotene at various stages of growth in various strains of *Phycomyces blakesleeanus*. *P. blakesleeanus* (-), $\circ$ — $\circ$; *P. blakesleeanus* var. *piloboloides* (-), $\bullet$ — $\bullet$; *P. blakesleeanus* mutant *pallens* (-), $\bullet$ — $\bullet$.

Fig. 2. The concentration of β -carotene at various stages of growth in various strains of *Phycomyces blakesleeanus* and in *Phycomyces nitens* (+). (The curve for the parent strain is repeated in this figure for ease of comparison.) *P. blakesleeanus* (-), $\blacksquare$ — $\blacksquare$; *P. blakesleeanus* var. *piloboloides* (+), $\circ$ — $\circ$; *P. blakesleeanus* mutant *gracilis* (+), $\circ$ — $\circ$; *P. blakesleeanus* mutant *pallens* (-), $\bullet$ — $\bullet$; *P. nitens* (+), $\bullet$ — $\bullet$.

much greater than in every other strain examined (Fig. 2). Lipid synthesis was essentially the same in all the (+) strains and did not differ from that in the (-) strains. The same conclusions apply to dry-weight productions except in the case of *P. nitens*. The normal dry-weight production by this strain in an 8 oz. medicine bottle is about 65 mg., whilst all the other strains produce between 80 and 100 mg.

DISCUSSION

All the morphological mutants of *P. blakesleeanus* examined, with the exception of *P. blakesleeanus* mutant *gracilis* (+), produce the same polyenes in the same amounts as do the (+) and (-) strains of the parent. From the point of view of helping to provide insight into the intermediary steps in the biogenesis of polyenes (in particular β -carotene), they must be considered as of no particular value. The mutant *gracilis* (+), however, falls out of line in two respects: it synthesizes as much carotene as other (-) strains (i.e. about twice as much as the normal (+) strains) and it does so at a very much greater rate. There is, however, no difference in the type of polyenes synthesized or in their relative amounts.

Carotene production in the parent strain is comparatively small during the growth of the mycelium, the greater part being synthesized after growth is complete (Goodwin & Willmer, 1952). As an explanation of this it has been suggested that carotene is synthesized from the products of the anaerobic dissimilation of glucose, the opportunities for this type of metabolism being greatest when the mycelium is completely formed as a mat covering the surface of a static culture; it is also possible that the demand for carotene precursors is only fully satisfied when the fungus is fully grown. Whatever the final explanation, it is clear from Fig. 2 that in *gracilis* (+) the situation is different. There are not two rates of carotenogenesis according to whether the fungus is growing or not. In this strain the rate of synthesis is almost exactly the same as the rate of growth. When more is known about the details of carotene synthesis, it will obviously be of interest to re-examine *gracilis* (+), but at the present state of knowledge, it is not likely to be of any more value than the parent. The (+) and (-) strains of the

Phycomycetes are considered to be the two sexual forms. Apart from their fundamental property of conjugating to form zygospores, the two forms are often characterized by their ability to synthesize different amounts of carotene (see Goodwin, 1950). Whether this is related to their sexual reactions or is purely fortuitous is not known. The present series of experiments does little to provide an answer to this problem. All the strains examined, except *gracilis* (+), conform to the general observation that the (-) strains produce about twice as much carotene as do the (+) strains. As previously stated, in this connexion *gracilis* (+) behaves as a (-) strain. It is observations of this type which make it doubtful that carotene production has any direct function in the reproduction of the Phycomycetes. In this connexion it would have been very interesting to have examined *gracilis* (-), but apparently this strain has not yet been isolated.

SUMMARY

1. The growth, lipid and carotene production of the following have been compared with those of *Phycomyces blakesleeanus* (-): *P. blakesleeanus* var. *piloboloides* (+ and - strains), *P. blakesleeanus* mutant *gracilis* (+), *P. blakesleeanus* mutant *patens* (-) and *P. nitens* (+).
2. Lipid production was the same in all; growth was the same in all but *P. nitens*, which produced about 70% of the dry weight obtained with the others.
3. Carotene synthesis in all the (-) strains was the same; in all the (+) strains except *gracilis* (+), carotene synthesis was about one-half of that in the (-) strain. The *gracilis* (+) mutant produced as much as a normal (-) strain.
4. The rate of synthesis of carotene was the same in all except *gracilis* (+), in which it was much more rapid.
5. The same polyenes occurred in all the mutants and in the same relative amounts.

We wish to thank Prof. R. A. Morton, F.R.S., for his continued interest in this work, which was carried out during the tenure (by L. A. G.) of a University of Liverpool Fellowship. Thanks are also due to the Medical Research Council for a grant towards laboratory expenses.

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