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Phenol Oxidases Produced by Mutant and Wild-Type Strains of *Aspergillus nidulans*

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A survey has been made of the occurrence of phenol oxidase in wild-type and mutant strains of *Aspergillus nidulans*. Certain phenol oxidases are thought to be involved in melanin synthesis, and therefore mutant strains have been isolated with abnormal melanin synthesis.

In strain BWB224, our standard strain, five bands of dihydroxyphenylalanine oxidase activity or diphenol oxidase activity can be detected by polyacrylamide disc electrophoresis. Six bands have been detected in a mutant strain. Several other workers have described heterogeneity in phenol oxidases in fungi (Carter & Bull, 1968; Esser, 1963; Horowitz *et al.* 1961; Jolley, 1966).

Changes in the phenol oxidase patterns throughout growth have been followed in batch culture. During active growth, the number of phenol oxidase types increases, reaching a maximum in the stationary phase. During autolysis, when melanin is being deposited in the walls, no new phenol oxidases appear.

Differential centrifugation has shown that specific phenol oxidase bands are associated with different particulate fractions, but all phenol oxidases can be seen in the soluble fraction. The cellular distributions of these enzymes at different stages in the life cycle have also been compared. Although the total number of phenol oxidase types present in the lag, exponential, stationary and autolytic phases differs, the type of distribution is similar. Only one or two phenol oxidases are present in low-speed fractions (up to 12000g). All but one type occurs in the high-speed fraction (100000g), whereas all types can be detected in the final supernatant.

After incubation at 50° for 1 min., one phenol oxidase is inactivated in samples from each stage in the growth cycle. Further characterization of the *A. nidulans* phenol oxidases by substrate and inhibitor specificities should indicate their inter-relationships.

Parameters of growth, morphology, phenol oxidases and melanin pigmentation have been compared in a variety of wild-type isolates and

mutants. Fewer phenol oxidase types are found in a highly melanized mutant, whereas up to six bands appear in a mutant devoid of melanin. The wild-types show a range of phenol oxidase patterns, from which it may be possible to classify them into groups.

Both mutants have been assigned to linkage group VII, and an attempt to map them further is in progress.

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The Effect of 3-Deoxy-3-fluoro-D-glucose, a Non-metabolizable Sugar, on the Metabolism of *Saccharomyces cerevisiae*

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On the basis of extent of oxygen uptake, carbon dioxide production and the activity of 3-FG* in various enzyme systems, Brunt & Taylor (1967) concluded that 3-FG was metabolized by yeast. This conclusion is in disagreement with our findings.

We find that: (1) 3-FG is not a growth substrate for *Saccharomyces cerevisiae* (strain 229, National Collection of Yeast Cultures); (2) when present in equimolar amount with glucose, 3-FG does not affect the growth of the yeast in any way (lag phase, doubling time or growth yield); (3) 3-FG does not disappear from the medium. We developed an assay for 3-FG based on its oxidation by glucose oxidase, the rate of which was directly proportional to the concentration of 3-FG. The rate of oxidation was much less (0.3%) than the initial rate for glucose under the same conditions of assay.

When 3-FG was added to yeast cells (from glucose-limited cultures) in Warburg respirometers the respiration rate increased up to sixfold. However, again 3-FG did not disappear to a significant extent from the flasks. The source of the substrate for respiration was investigated with yeast cells that had ¹⁴C-labelled carbon (the cells were grown on uniformly labelled glucose). By this means it was found that the carbon dioxide evolved when 3-FG was added to a resting yeast suspension was exclusively of cellular origin and not from 3-FG.

* Abbreviation: 3-FG, 3-deoxy-3-fluoro-D-glucose.

It was also found by means of the ^{14}C -labelled yeast that glucose, like 3-FG, stimulated endogenous metabolism so that about one-third of the carbon dioxide evolved was of cellular origin.

The observed increase in oxygen uptake and carbon dioxide production after the addition of 3-FG to resting cells of *Saccharomyces cerevisiae* must be accounted for by an increase in endogenous metabolism.

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Photosynthetic-Carbon-Cycle Enzyme Activities in Leaves of a Chlorophyll-less *Phaseolus vulgaris* Plant

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Chlorophyll-less plants of *Phaseolus vulgaris* cultivar Alabaster seem to occur quite rarely. When such a plant was found in a batch of seedlings that had been allowed to green in natural daylight in July, the primary leaves were taken for electron microscopy and the assay of enzyme activities (Bradbeer, 1969) at the stage at which the leaves appeared to have reached their maximum size, fresh wt. 133 mg./leaf. No indication of the onset of senescence in these leaves was obtained from their protein content, 7.02 mg./leaf, or from electron microscopy. The electron micrographs revealed the presence of plastid-like organelles that did not show any internal lamellar structure.

The chlorophyll-less leaves have been compared with the etiolated leaves of plants grown for 14 days in the dark (fresh wt. 35 mg., protein content 1.93 mg.) and also with the fully greened leaves of plants grown for 14 days in darkness followed by 5 days of continuous illumination at $500 \mu\text{w}/\text{cm}^2$ (fresh wt. 133 mg., protein 4.72 mg.). The etiolated leaves contained all of the investigated enzymes of the photosynthetic carbon cycle but the activities were very much lower than those of fully greened leaves.

The three photosynthetic-carbon-cycle enzymes that are confined to the chloroplasts, namely ribulose diphosphate carboxylase, phosphoribulokinase and NADP-linked triose phosphate dehydrogenase, were present in the chlorophyll-less leaves at about the same activities as in etiolated leaves.

Four enzymes, ribose phosphate isomerase, phosphoglycerate kinase, fructose diphosphate aldolase and transketolase, showed activities almost

midway between those of etiolated and fully greened leaves. These enzymes are all implicated in the photosynthetic carbon cycle but they are not confined to the chloroplasts.

Triose phosphate isomerase, NAD-linked triose phosphate dehydrogenase and phosphopyruvate carboxylase occurred in chlorophyll-less leaves at about twice the activity found in fully greened leaves. Of these three enzymes only triose phosphate isomerase appears to be a component of the photosynthetic carbon cycle in beans, and this enzyme and NAD-linked triose phosphate dehydrogenase occur both inside and outside of the chloroplasts.

Chlorophyll-less plants of *Vicia faba* have been reported to lack chloroplast ribosomes (T. A. Dyer, R. Miller & A. D. Greenwood, unpublished work), but no information on this point is available for *Phaseolus*.

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The Purification and some Properties of a Monohaloacetate Dehalogenase from *Pseudomonas dehalogenans* N.C.I.B. 9061

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Several micro-organisms can eliminate halogen atoms from halogenated aliphatic or aromatic compounds; such reactions play an important part in the breakdown of halogenated herbicides in the soil. The enzymic release of the halogen atom of monohaloacetate as inorganic halide has been studied in cell-free extracts (Davies & Evans, 1962) and with partially purified enzymes (Goldman, 1965; Goldman & Milne, 1966; Goldman, Milne & Keister, 1968).

Pseudomonas dehalogenans N.C.I.B. 9061 was grown aerobically at 28–30° on a mineral salts medium containing monochloroacetate (0.37%) as sole carbon source. Cell-free extracts, prepared by ultrasonic disintegration of the harvested cells, contained an enzyme that catalysed the release of inorganic chloride from monochloroacetate. This enzyme was assayed by conductimetric titration with 1 mM-AgNO₃ of the chloride released after 10 min. reaction at pH 9.0 and 30°.

In agreement with the other haloacetate dehalogenases the enzyme had a pH optimum of 9.5; below pH 5.0 activity was irreversibly lost. Dialysis for 10 hr. at pH values between 7.0 and 10.0 produced no significant loss of activity. At pH 7.0 the enzyme was stable for at least 48 hr. at ambient temperatures, and indefinitely at 4°; the activity