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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.