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and V.G. Del Vecchio. A rapid assay for  
tyrosinase activity.

The enzyme, tyrosinase, has been extensively used  
in studies of the genetic control of enzyme synthesis.  
It is widely distributed in plant, animal, fungal and  
bacterial cells. Tyrosinase catalyzes the oxidation

of tyrosine or dihydroxyphenylalanine (dopa) in the biosynthesis of melanin. The enzyme, in the presence  
of substrate results in the formation of an insoluble black pigment which can be quantitatively assayed  
spectrophotometrically.

A rapid, simplified method which can be semi-quantitated has been used for the detection of this  
enzyme in extracts of Neurospora crassa and Bacillus subtilis. This method, a modification of one develop-  
ed by Cooper and Brown (1956) does not require extensive instrumentation and is useful for rapid screening  
procedures, since extracts of several strains can be tested and compared simultaneously.

Essentially, the method consists of adding extracts of disrupted cells to wells cut in an agar plate into  
which substrate has been incorporated. The presence of the enzyme is evidenced by a ring of black pig-  
ment which precipitates in the agar surrounding the well. Preliminary results indicate that this assay may  
be valuable for semi-quantitative determinations. The size of the black ring and the density of the pigment  
granules surrounding the well is proportional to the concentration of the extract used in the test and to the  
degree of purification of the enzyme. The prolonged lag period usually encountered when tyrosine is used  
as the substrate can be minimized by using partially purified extracts and by incorporating a low concen-  
tration of dopa along with the tyrosine in the agar. The enzyme has an absolute requirement for copper.

The agar used in the petri dishes must be carefully prepared to avoid the auto-oxidation of the dopa.  
Two solutions are made up separately and combined just prior to pouring the plates. 8.0 g. of lanager are  
added to 500.0 ml of phosphate buffered saline (0.85% NaCl buffered at pH 6.8 with 0.5 M phosphate)  
and autoclaved. A second solution containing a 1:3 ratio of dopa to tyrosine is prepared by adding 0.1 g.  
dopa and 0.3 g. tyrosine to 500 ml of distilled water which was made acidic with 4 drops of 6 N HCl.  
The dopa-tyrosine solution was added to the buffer when the latter had cooled to 65-70°C. 30 ml aliquots  
were dispensed to petri dishes and allowed to solidify. Wells were cut in the agar using standard tem-  
plates available for the preparation of agar-diffusion plates. The bottom of each well was sealed with a  
drop of agar prior to use.

The enzyme extracts from Bacillus subtilis require both the presence of copper and dopa in agar for  
good results. The catalytic activity of the Neurospora enzyme can be demonstrated without the addition  
of exogenous copper. (Supported in part by a U.S.P.H.S. Grant, N.I.H. A-5376, 2 Dr. K.E. Fuscaldo)  
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