Fungal Genetics Reports

Volume 3 Article 8

A rapid assay for tyrosinase activity

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Recommended Citation

Fuscaldo, K. E., W.K. Kaczmarczyk, and V.G. Del Vecchio (1963) "A rapid assay for tyrosinase activity," *Fungal Genetics Reports*: Vol. 3, Article 8. https://doi.org/10.4148/1941-4765.2154

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Abstract

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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 developed 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 pigment 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 concentration 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 Lonager 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 templates 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) ---Department of Biology, St. John's University, Jamaica, New York, U.S.A.