A simple method for the induction of high levels of tyrosinase activity

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Abstract
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absorbancy at 340 μg is recorded for at least 3 min. An appropriate final dilution of enzyme from mycelial extracts containing about 5 mg protein is generally 2.5 × 10⁻⁴. One unit of enzyme is defined as that amount of protein catalyzing the oxidation of one μmole of NADH in the first minute of reaction. The initial reaction velocity is linear up to 5 min and proportional to protein concentration when the measured activity is less than 1/800 μg unit (corresponding to an absorbancy change of less than 0.08 per minute). Specific activity is expressed as units per mg of protein. - - - Department of Biologic, 1 Sciences, Stanford University, Stanford, California.


The strain used, m-25, is microconidiating with a very compact colonial growth. Conidia were routinely irradiated (in a concentration of 10⁷/ml) with UV from a low-pressure Hg-lamp. Irradiation with doses to give 3-6% survival was carried out in the dark to prevent photoreactivation. The irradiated conidia were immediately diluted in Fries' minimal medium and plated on the surface of minimal agar medium. Discs of thin terylene net (the meshes about 0.4 mm2) were, after plating, superimposed tightly onto the surface of the medium. The colonies are, after 5-7 days of growth in the dark at 28°C, attached to the net and can be partly removed with the latter, leaving a replica on the plate. Enzymatic testing can then be performed on one of these replicas, leaving the other for isolations.

A 1.5% solution of sodium perborate (NaBO₃, 3H₂O, H₂O₂) was used as a substrate for the catalytic reaction, according to the method described by Feinstein (1949 J. Biol. Chem. 160: 1197). The solution was adjusted to pH 6.7 and made semi-solid with 0.2% agar. In a typical experiment the net was removed and the remaining parts of the colonies flooded with the substrate. A distinct production of gas from the individual colonies can be seen after a few seconds. Bubbles of gas, originating from oxygen evolving by the reaction: 2 H₂O₂ → 2 H₂O + O₂, collect around each colony owing to the semi-solid substrate. Colonies producing no, or very small amounts of oxygen were isolated from the replica on the net, or, in some instances when the colony grew submerged in the medium, directly on the dish.

About 60,000 colonies have been tested according to this method. 30 colonies were isolated as quasi mutants. None of these proved to be entirely devoid of catalytic activity by a secondary assay, but some showed a markedly reduced production of gas as compared to the original strain. It is, in this connection, still open question whether or not a mutant is viable if completely devoid of catalase activity, since this enzyme is generally acting as a detoxifier by removing H₂O₂ produced during metabolic reactions.

Some other methods were attempted, for instance using a 1% solution of H₂O₂ as substrate for the enzymatic reaction, and testing the colonies on the net. It was also tried to absorb KJ-starch or KMnO₄ into filter paper and use these preparations as indicators for the presence or absence of H₂O₂. The net with colonies was dipped into a solution of H₂O₂, then taken out again and allowed to react for 5 minutes. Absorbed H₂O₂ is during this period decomposed by catalase. Normal colonies, therefore, show no color reaction when subsequently placed in contact with the H₂O₂ indicator, while a catalaseless mutant should be revealed by a positive reaction for H₂O₂. The latter mentioned methods, however, seem to be less reliable in practical use than the gas developing assay. - - - Institute of Genetics, University of Stockholm and Institute of Physiological Botany, University of Uppsala, Uppsala, Sweden.

Poll, M. L., G. Horn, M. Fling, and N. H. Horowitz. A simple method for the induction of high levels of tyrosinase activity. Laboratory of not requiring a change of medium during the run. It is about equally effective in inducing strains 69-1 113a (T²) and 4-137a (T²). The data reported here ore for these two strains.

Induction in flasks: 125 ml Erlenmeyer flasks are prepared with 20 ml of 1/2 strength Vogel's medium N containing 1/2% sucrose. Each flask is inoculated with one drop of a heavy conidial suspension and placed at 25°C for 48 hours. Three mg of either DL-ethionine or D-phenylalanine are then added and the flasks are placed on a reciprocal shaker and gently shaken at 25°C for two days. The flasks yield from 60 to 120 mg wet weight of mycelium with an activity of from 250 to 300 Enzyme Commission units per gram wet weight as determined by the method of Fling et al. (1963 J. Biol. Chem. 238: 2045). Wild type strains other than 4-69-113a and 4-137a have been induced by this procedure although many strains require different levels of ethionine or D-phenylalanine for optimal induction than the 150 μg per ml used above. Still to be determined is whether any ethionine is incorporated into the enzyme induced by this method.

Induction in carboys: Two and one-half gallon Pyrex carboys are filled with 8 liters of 1/2 strength Vogel's medium N, 1/2% sucrose, and equipped with a single glass tube for aeration and agitation. Each carboy is inoculated with about 10⁷ conidia and the flow of water-saturated air is adjusted to about 5 liters/minute. They are generally kept in the dark. After about 48 hours, 1.2 g of DL-ethionine are added. Starting at about 2 1/2 days after the addition of the ethionine, samples are removed aseptically from each carboy periodically and assayed in order to determine the time of the maximum level of enzyme activity. Maximum activity is generally attained about 3 to 5 days after addition of the ethionine. Slowly inducing carboys can often be speeded by increasing the air flow. Usually 50-80 g of mycelium are obtained per carboy induced to a level of between 120-400 Enzyme Commission units per gram wet weight of mycelium. D-phenylalanine can also be used for induction in carboys although optimal conditions for maximum activity have not been determined.

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