

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

M. L. Pall

G. Horn

M. Fling

N. H. Horowitz

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Abstract

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laboratory of not requiring a change of medium during the run. It is about equally effective in inducing strains 69-1113a (T^S) and 4-137a (T^L). The data reported here are 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 500 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 69-1113a 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 eight 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 10⁹ 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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A technique for the induction of *Neurospora* tyrosinase to high levels of activity both on a small scale (in flasks) and on a large scale (in carboys) has been developed. This procedure has the advantage over the starvation method previously used in this