Fungal Genetics Reports

Volume 8 Article 25

A method for detection of catalase mutants

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Abstract
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Olsson, L. and H. G. Kølmark. A method for detection of catalase mutants.

A screening method has been developed with the aim of detecting mutants of Neurospora crassa deficient in activity of the enzyme catalase.

The strain used, m-25, is microconidiating, with a very compact colonial growth. Conidia were routinely irradiated (in a concentration of 10^7/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 ($NaBO_2.3H_2O.H_2O_2$) was used as a substrate for the catalytic reaction, according to the method described by Feinstein (1949 J. Biol. Chem. 180: 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: $2\ H2O2 \xrightarrow{\text{catalase}} 2\ H2O + O_2$, 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 hove been tested according to this technique. 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 on 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 H202 produced during metabolic reactions.

Some other methods were attempted, for instance using a 1% solution of H202 as substrate for the enzymatic reaction, and testing the colonies on the net. It was also tried to absorb KJ-starch or $KMnO_4$ into filter paper and use these preparations as indicators for the presence or absence of H_2O_2. The net with colonies was dipped into a solution of $H20_2$, then taken out again and allowed to react for 5 minutes. Absorbed H202 is during this period decomposed by catalase. Normal colonies, therefore show no color reaction when subsequently placed in contact with the H_2O_2 indicator, while a cataloseless mutant should be revealed by a positive reaction for H_2O_2. 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 Uppsola, Uppsala, Sweden.