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Color Reaction Streak Test for Catalase-Positive Microorganisms

JACOB S. HANKER* AND ALBERT N. RABIN

Dental Research Center, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

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A stable purple color results when a reagent solution is applied to a smear of catalase-positive organisms streaked on a glass slide.

Catalase assays have practical application in microbial studies in that catalase determination is a useful method for differentiating most aerobic bacteria from anaerobes and facultative anaerobes. Anaerobes are not able to decompose H_2O_2 and are classified as catalase negative, whereas most aerobes, with the exception of *Streptococcus*, contain catalase and breakdown H_2O_2.

Most catalase assays performed are based on the catalatic action of the enzyme, which results in the decomposition of H_2O_2 and the liberation of O_2 (Fig. 1a). Tests dependent on the catalatic activity are potentially hazardous due to the production of bacteria-laden aerosols by liberated oxygen (1). Such tests, moreover, must be monitored during the course of the procedure as no record is left of the results once effervescence subsides.

Color reactions have been used to demonstrate the peroxidatic activity (Fig. 1b) of catalase (2). In these reactions, catalase mediates the oxidation of certain phenols and aromatic amines by H_2O_2 without the liberation of oxygen, but the colors produced are not satisfactory for routine use.

A series of 30 compounds was tested individually and in combination to determine which of these substances would form the most intensely colored compounds when oxidation by H_2O_2 is catalyzed by the peroxidatic action of catalase.

a $$2H_2O_2 \xrightarrow[\text{Action}]{\text{Catalatic}} 2H_2O + O_2\uparrow$$

b $$H_2O_2 + H_2A \xrightarrow[\text{Action}]{\text{Peroxidatic}} 2H_2O + A$$

FIG. 1. *The catalatic and peroxidatic reactions of the enzyme catalase. H_2A denotes a colorless aromatic amine or phenol which is oxidized to form a colored compound signified as A in the reaction. (a) Catalatic (catalase-like) action of the enzyme and (b) its peroxidatic (peroxidase-like) activity.*

It was found that a catalase detection reagent solution with a low blank could be prepared by adding the following ingredients in order: dopamine (Polysciences, Inc., Warrington, Pa.) (20 mg/ml in 0.2 M phosphate buffer, pH 8.0), 1 ml; *p*-phenylenediamine dihydrochloride (Polysciences, Inc.) (1 mg/ml in 0.2 M phosphate buffer pH 8.0), 1 ml; H_2O_2 (3%), 2 ml; dimethyl sulfoxide (Polysciences, Inc.), 1 ml. When this reagent was peroxidatically oxidized, a highly colored purple product was formed that was stable for hours.

Representative samples of aerobic and anaerobic bacteria, as well as facultative anaerobes, were tested with this reagent for catalase activity. Pure cultures of aerobic bacteria were grown on Trypticase soy agar, whereas pure anaerobes and facultative anaerobes were grown on blood agar in anaerobic jars. The cultures were incubated for 24 h at 37 C. The bacteria were then streaked in duplicate on glass slides and the smears were allowed to dry. One drop of 3% H_2O_2 was added to one slide, and the evolution of oxygen bubbles was considered indicative of catalase activity. On the second smear, one drop of catalase detection reagent solution was added. If the bacteria were catalase positive, a purple-colored compound would form on the slide.

Table 1 shows the results of testing a variety of bacteria for catalase activity. Note the correspondence of effervescence and color formation. The test is especially useful to differentiate staphylococci from streptococci.

The objective of this project was to produce a simple, rapid test to demonstrate catalase activity in bacteria by the peroxidatic action of the enzyme. Enzyme activity is demonstrable within seconds by this colorimetric procedure, and the colored oxidation products last for days so that data can be rechecked. This colorimetric procedure is safe in that the investigator is not exposed to an aerosol of pathogenic bacteria.

TABLE 1. *Direct visual estimation of bacterial catalase activity by streak test*

Organisms	Effervescence[a]	Color[b]
Aerobic		
Enterobacter aerogenes	+++	++++
Bacillus cereus	+++	++++
B. megaterium	+	+
Escherichia coli	+++	++++
Micrococcus lutea	++	++
Pseudomonas aeruginosa	+++	++++
Staphylococcus epidermidis	+++	++++
S. aureus HR	+++	++++
S. aureus NCMH	+++	++++
S. aureus PR101	+++	++++
S. aureus PR102	+++	++++
S. aureus Vac	+++	++++
Streptococcus faecalis G	0	0
S. faecalis HR	0	0
S. faecalis NCMH	0	0
S. pyogenes	0	0
Anaerobic and facultative anaerobic		
Bacteroides fragilis	0	0
B. melaninogenicus	0	0
Streptococcus agalactiae	0	0
S. lactis	0	0
S. mutans	0	0

[a] Catalatic (catalase-like) activity, decomposition of H_2O_2.

[b] Peroxidatic (peroxidase-like) activity, oxidation of dopamine-*p*-phenylenediamine reagent.

The results of the peroxidatic assay performed on representative samples of bacteria are consistent with existing data on catalase activity. It is anticipated that this new colorimetric procedure will be more satisfactory than existing methods for determining catalase activity in bacteria.

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