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The catalase-aminotriazole assay for determination of hydrogen peroxide apparently cannot be used for measuring hydrogen peroxide production in crude preparations from wood decay fungi because of materials in the crude preparations that interfere with the test.

The catalase-aminotriazole (C-At) assay has been used to measure small amounts of H₂O₂ production by bacteria, fungi, and isolated oxidases (2, 5, 6, 8). This method is based upon the inhibition of catalase by aminotriazole in the presence of H₂O₂. The rate of catalase inactivation provides an estimation of the H₂O₂ production rate. However, the C-At system actually measures the continuous addition of small amounts of H₂O₂ (2) and is therefore very sensitive.

Koenigs (6), working with several wood-rotting fungi, was unable to detect H₂O₂ production by these fungi with a titanium reagent (3), but he was able to detect H₂O₂ production from glucose with the C-At assay. His explanation for the inability to detect H₂O₂ with the titanium reagent was that in liquid cultures the H₂O₂ concentrations were very low or that H₂O₂ may be present only for a short time because of destruction by peroxidase or catalase. Since the C-At system involves a biochemical trap, the system yields positive indications of H₂O₂ production in the presence of peroxidase or catalase. However, catalase was not detected by Koenigs (6) in any of the fungi, and peroxidase was not found in the brown-rot fungi. Therefore, these enzymes would not limit H₂O₂ accumulation. We also were unable to detect catalase and peroxidase in several brown-rot fungi (unpublished data).

Any products produced by the microorganisms that inactivate catalase would result in a positive indication of H₂O₂ with the C-At assay. Wood-decay fungi produce numerous organic acids (10), several of which are reported to inhibit catalase (7). The presence of these acids, or perhaps other materials, could interfere with the accurate determination of H₂O₂ with the C-At method. Although several controls were used in Koenigs' work, he does not report the use of heat-inactivated controls (5, 6). Heating destroys H₂O₂ and H₂O₂-producing enzymes.

To determine whether crude preparations from brown-rot and white-rot fungi contain materials that interfere with the measurement of H₂O₂ by the C-At method, assays were conducted with both heated and unheated preparations. The fungi were grown in stationary culture with 30 ml of medium consisting of 2% malt extract, 2% glucose, and 0.1% peptone (5). Flasks were inoculated with 11 fungi (Table 1) and incubated in the dark for 21 days. Hemlock (Tsuga heterophylla (Raf.) Sang.) sawdust was decayed by the American Society for Testing and Materials soil block procedure (1). Samples of sawdust (0.5 g) were placed on inoculated pine feeder strips in soil block bottles for 30 days.

The C-At assay was that of Cohen and Somerson (2) as modified by Koenigs (5, 6), with glucose added to support production of H₂O₂ (C-At-G). Catalase was purchased from Worthington Diagnostics, Freehold, N.J. (63,863 U/ml), and 3-amino-1,2,4-triazole was purchased from Sigma Chemical Co., St. Louis, Mo. After 21 days, mycelium and filtrates from liquid cultures were separated. C-At-G incubation medium (10 ml) was added to the mycelium. Filtrate (1 ml) was added to 5 ml of C-At-G. C-At-G (8 ml) was added to decayed sawdust after removal from soil block bottles. Duplicate filtrates, mycelium, and decayed sawdust were heated at 212°C for 30 min and assayed with C-At-G in the same manner as the unheated preparations. After 1 h, 0.5-ml samples were withdrawn from each incubation medium and added to 5 ml of 0.006 M H₂O₂ solution, and inactivation of catalase was determined by titration with 0.01 N permanganate.

The formation of H₂O₂ from glucose was also determined by the use of o-dianisidine (9). The malt-peptone-glucose liquid medium was not
used for these assays because of background color that interfered with the assays. Therefore, the fungi were grown on a medium consisting of basal salts (4) and 1% glucose. Filtrates were assayed for H₂O₂-producing activity after 2 weeks by incubating 1 ml of filtrate with 4 ml of o-dianisidine–peroxidase–glucose reagent, pH 5.0. At the end of the incubation period (maximum, 24 h), 0.2 ml of 4 N HCl was added to terminate the reaction. Absorbance was read at 460 nm.

The o-dianisidine assay detected H₂O₂ production from glucose by filtrates of the white-rot fungi *Coriolus versicolor*, *Phellinus ferrugineofuscus*, and *Sponigipellis unicolor*. Only filtrates of one brown-rot fungus, *Leucogyrophana olivascens*, indicated H₂O₂ production from glucose.

Table 1 shows the percent inactivation of catalase in the presence of aminotriazole by heated and nonheated filtrates and mycelium taken from liquid cultures of the wood-decay fungi. The effect of heated and nonheated sawdust decayed by several brown-rot fungi on catalase activity with aminotriazole is reported in Table 2.

In the presence of aminotriazole, heated and nonheated filtrates, mycelium, and decayed wood from the brown-rot fungi inhibited catalase activity in a similar fashion. The heated and nonheated preparations from the white-rot fungi had a more variable effect on catalase activity. This variation could be due to the production of peroxidase or H₂O₂-producing oxidase by the white-rot fungi. The brown-rot fungi did not produce peroxidase, and only *L. olivascens* produced an extracellular H₂O₂-producing oxidase. However, the heated filtrates and mycelium from the white-rot fungi caused at least some inhibition of catalase in the presence of aminotriazole, with the exception of *C. versicolor* (neither heated nor unheated preparations had inhibitory action).

These results indicate that wood-decay fungi produce materials that inhibit catalase and, thereby, interfere with the accurate determination of H₂O₂ with the C-At assay. Thus, enzyme preparations from wood-decay fungi should be purified to remove catalase inhibitors when the C-At method is used to measure H₂O₂ production.

**LITERATURE CITED**


