Oxidation of Alcohols by *Botrytis cinerea*¹

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Crude cell-free preparations of *Botrytis cinerea* were found to oxidize straight-chain primary alcohols (except methanol), aromatic primary alcohols, and unsaturated primary alcohols. The resulting products were the corresponding aldehydes and an equal molar quantity of hydrogen peroxide.

Alcohol oxidases of different substrate specificity have been obtained from many microorganisms (1, 3, 5). DeJong and co-workers (2) have found a glucose dehydrogenase and a phenol oxidase in extracts of lyophilized *Botrytis cinerea*. This fungus, parasitic to ornamental and crop plants (6), was shown in our laboratories to have alcohol-oxidizing activity which is described in this paper.

Spores of *B. cinerea* Lilly M45-289 were incubated aerobically at 30°C on a rotary shaker (250 rev/min) for 48 hr in a medium composed of 2% glucose, 2% malt extract (BBL), and 0.1% yeast extract (Difco). The resulting mycelial growth was suspended in water, blended for 20 sec, and then sonically treated in a 10-kc Raytheon oscillator for 60 min. After the mycelial debris was removed by centrifugation, the supernatant was subjected to ammonium sulfate precipitation. The precipitate obtained between 0.6 to 0.9 ammonium sulfate saturation was dialyzed against 0.05 M potassium phosphate buffer (pH 6.0) for 18 hr at 4°C. The solution of partially purified oxidase was then freeze-dried. No effort was made to determine if more than one alcohol oxidase was present in the preparation.

The oxidizing activity of the freeze-dried preparation was determined by reacting it with substrate alcohols (reagent grade) and measuring the quantity of hydrogen peroxide produced. A chemical assay for hydrogen peroxide was used by employing o-dianisidine, as described by Janssen and Ruelius (4). The color intensity of the o-dianisidine-H₂O₂ product was measured spectrophotometrically and converted to moles of peroxide by a standard curve (30% hydrogen peroxide, Baker).

The optimum pH and temperature for the oxidase activity were measured for the oxidation of ethanol. Maximum activity was demonstrated between 48 and 53°C with a pH range of 5.2 to 5.8.

The stoichiometry of the oxidation of benzyl alcohol by the oxidase preparation to benzaldehyde and hydrogen peroxide was determined. To a solution of 3 mg of oxidase at 25°C was added 4.8 mg of benzyl alcohol (J. T. Baker Chemical Co., reagent). Sequential samples of the reaction were assayed for hydrogen peroxide by the o-dianisidine procedure. The molar concentrations of benzyl alcohol and benzaldehyde in the samples were determined by gas chromatography (Porapac-type Q column). One mole of hydrogen peroxide and 1 mole of benzaldehyde were obtained from each mole of benzyl alcohol oxidized. No benzoic acid or phenolic products were detected by gas chromatography.

The reactivity of the crude *B. cinerea* oxidase preparation with substrate alcohols is shown in Table 1. The quantity of hydrogen peroxide (2.4 × 10⁻⁶ mmoles), obtained by the oxidation of each substrate in the presence of the oxidase, is shown in Table 1.

Table 1. Relative reactivity of alcohols with the crude *Botrytis cinerea* oxidase preparation

| Substrate       | Relative reactivity |
|-----------------|---------------------|
| Ethanol         | 1.0                 |
| Methanol        | 0.0                 |
| 1-Propanol      | 0.25                |
| 1-Butanol       | 19.0                |
| 1-Pentanol      | 38.0                |
| 1-Hexanol       | 137                 |
| 1-Heptanol      | 126                 |
| 1-Pentyl alcohol| 0.0                 |
| 2,2-Dimethyl-1-propanol | 0.0             |
| Isopropyl alcohol| 0.0                |
| Isobutyl alcohol| 0.0                 |
| Cyclohexanol    | 0.0                 |
| H₂C=CHCH₂OH     | 122                 |
| H₂CC=CHCH₂OH    | 1,302               |
| HCC=CHCH₂OH     | 3.0                 |

Table continued on p. 551

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of 0.1 mmole of ethanol with 1.0 mg of oxidase preparation at 25 C for 5 min, was given the value of one. The activity of several substrate alcohols was determined over a range of alcohol concentrations to minimize inhibition of the oxidase activity at high substrate or product concentrations.

The results in Table 1 show that unsaturated and aromatic primary alcohols are better substrates for the crude B. cinerea oxidase(s) than aliphatic alcohols. Secondary and branched-chain primary alcohols were not oxidized. The monofunctional and branched primary alcohols listed in Table 1 are representative of the extensive number which were tested. Introduction of a polar group (halogen, amine, thiol, hydroxyl) completely inactivated primary alcohols as oxidase substrates. None of the numerous sterols, carbohydrates, or amino acids tested was oxidized by the crude B. cinerea oxidase preparation.

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