Formation of *p*-hydroxybenzoic acid from phenylacetic acid by *Poria weirii*

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Phenylacetic acid is known to inhibit growth of *Poria weirii* cultures at concentrations of 2.0 mM but not at 0.5 mM. In this study, the compound at the lower concentration was metabolized by the fungus by degradation to noninhibitory *p*-hydroxybenzoic acid and an unknown compound.

Phenylacetic acid is a phenolic substance that has been shown to inhibit significantly growth of *P. weirii* cultures at 2.0 mM concentration but to have no significant effect at a 0.5 mM concentration (Li *et al.* 1969). The study reported here was designed to determine if *P. weirii* metabolizes phenylacetic acid at the lower concentration.

Still cultures of isolate T-124 of *P. weirii*, used by Li *et al.* (1969), were grown at room temperature in 500-ml Erlenmeyer flasks containing 200 ml of modified Trione's (1964) liquid synthetic medium. Modifications included the following: (1) controls—20 g sucrose replaced with 10 g glucose; (2) phenylacetic acid treatment—0.5 mM phenylacetic acid added aseptically to the control medium. In addition, uninoculated flasks of both media modifications were included in the experiment as a check on stability of the components in the absence of the fungus. Each treatment was replicated three times.

Twenty-eight days after inoculation, the mycelium was filtered out from the medium of inoculated flasks. The filtrates and the solutions from the uninoculated treatments were adjusted to pH 2 with 0.1 N HCl and extracted three times with ethyl ether. The ether extracts were dried on a flash evaporator, redissolved in 4 ml ethanol, and chromatographed by an ascending, one-dimensional system on duplicate thin-layer plates of microcrystalline cellulose containing 4% ZS Super inorganic fluorescent dye. After development in isopropyl alcohol : NH_4OH : H_2O (8:1:1), plates were examined under ultraviolet (uv.) light (254 mμ) and the uv.-absorbing spots were marked. Bromocresol green indicator was sprayed on one duplicate plate of each set to detect phenylacetic acid. The other duplicate plate of each set was sprayed with diazotized *p*-nitroaniline followed by 2 N NaOH to detect phenolic substances. Duplicate spots on every plate, covered during the spraying, were eluted with methanol and their uv.-absorption spectra determined with a Perkin-Elmer double-beam spectrophotometer. Identification of compounds from hR_f ($R_f \times 100$) values, spot color, and uv.-absorption spectrum was confirmed by comparison with authentic compounds treated by the same procedures. Particular attention was devoted to detection of hydroxyphenylacetic acids, since these have been reported as metabolites of phenylacetic acid for many microorganisms.

Results were identical for all replicates within each treatment. No phenolic substances appeared on chromatographs of media to which no phenylacetic acid had been added, whether inoculated with the fungus or not. Both inoculated and uninoculated phenylacetic acid treatments yielded residual phenylacetic acid plus an unknown with hR_f 6 and a pale yellowish-pink color after *p*-nitroaniline spraying. Only the inoculated phenylacetic acid treatment yielded *p*-hydroxybenzoic acid; and, only the uninoculated phenylacetic acid treatment yielded an unknown with hR_f 75 and a light orange color after *p*-nitroaniline spraying.

The metabolism of phenylacetic acid has been studied for some bacteria (e.g., Blakely *et al.* 1965) and numerous fungi in the Ascomycetes, Coelomycetes, Hyphomycetes, and Phycomycetes (e.g., Faulkner and Woodcock 1968; Kohmoto *et al.* 1970). In most cases, the organisms hydroxylated phenylacetic acid to one or more hydroxyphenylacetic acids. The only higher Basidiomycete included in these reports was the

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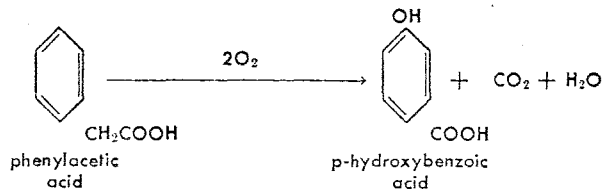
resupinate polypore, *Irpex lacteus* (Fr.) Fr., which failed to produce a monohydroxyphenylacetic acid (Kohmoto *et al.* 1970). *Poria weirii*, a resupinate polypore, also did not metabolize phenylacetic acid to hydroxyphenylacetic acids in our experiments.

The degradation of phenylacetic acid to *p*-hydroxybenzoic acid by *Poria weirii* can be visualized in the following way.



An intermediate step in this metabolic sequence may be represented by the unknown compound detected, which, by chromatographic comparison, was shown to be neither *p*-hydroxyphenylacetic acid nor benzoic acid. Li *et al.* (1969) demonstrated that *p*-hydroxybenzoic acid did not significantly affect growth of two isolates of *P. weirii* at concentrations up to 2.0 mM. The fungus is capable of detoxifying phenylacetic acid at relatively low concentrations, probably through oxidation and hydroxylation. Hydroxylation has been suggested as a detoxification mechanism of some fungi (Woodcock 1960).

The degradation of some phenylacetic acid in the absence of the fungus differed from that with the fungus present. The nonbiological breakdown of the compound reemphasizes the need for uninoculated controls in experiments of this kind.

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