**Poria weirii** as a Possible Commercial Source of Peroxidase

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**Poria weirii** produced peroxidase in yields amounting to 35% of those obtained from the same weight of horseradish roots. The three isozymes detected were distinct from those of horseradish.

Horseradish peroxidase is used to synthesize lignin and lignin-related model compounds (3, 11, 12). The enzyme is also produced extracellularly by white-rot (lignin-degrading) basidiomycetes (4, 6, 8) and may degrade wood. Thus, depending on experimental purposes, alternative sources of the enzyme are desirable.

Among wood-rotting fungi, *Poria weirii* Murr. synthesizes an unusually high amount of peroxidase (4). In this paper, 21 isolates of this fungus were evaluated for the production of this enzyme. One of them (strain ATCC 22570) was found to produce a peroxidase with isozymes distinct from the horseradish enzyme, and hence might be of commercial interest.

The isolates were cultured with and without 1% wood meal from loblolly pine (*Pinus taeda* L.) in triplicate flasks and harvested as previously described (4). At the end of the incubation period, the flask contents were pooled, filtered, and spectrophotometrically assayed in duplicate for peroxidase content (4). An enzyme unit is defined as an increase of 0.001 absorbance (A) unit/min. For comparison the enzyme was also extracted from horseradish roots (maintained in running water for 8 days before use) by grinding the tissue with sand and a pestle in a mortar at 4°C with 5 ml of 0.05 M phosphate buffer (pH 6.0) per g (wet weight); 15 ml of buffer was added per g of original weight. Crude extract was assayed after centrifugation at 10,000 × g for 10 min at 2°C. Dry weights of centrifuged solids were determined by drying at 65°C for 48 hr.

Four isolates producing the highest amounts of peroxidase were grown with and without 1% pine wood supplement (Table 1). The activity elaborated by isolate ATCC 22570 after 28 days of incubation with wood was 12,150 A units/g (dry weight); this amount is 12 to 33 times higher than that produced by the other three isolates cultivated under the same conditions, and is 35% of the amount extracted from horseradish roots (35,140 A units/g).

Proteins in culture filtrates and horseradish

### Table 1. Effect of 1% pine wood meal on peroxidase activity in four isolates of *Poria weirii* at 25°C

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Wood (1% w/v)</th>
<th>A/min/ml of culture filtrate</th>
<th>Total A/min/g of mycelial dry wt/flask*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>28 Days</td>
<td>35 Days</td>
</tr>
<tr>
<td>FP 91601-S (ATCC 14795)</td>
<td>−</td>
<td>13.2</td>
<td>9.9</td>
</tr>
<tr>
<td>T-91 (ATCC 22568)</td>
<td>+</td>
<td>14.6</td>
<td>5.6</td>
</tr>
<tr>
<td>T-103 (ATCC 22569)</td>
<td>+</td>
<td>7.9</td>
<td>11.7</td>
</tr>
<tr>
<td>T-154 (ATCC 22570)</td>
<td>−</td>
<td>6.7</td>
<td>9.5</td>
</tr>
</tbody>
</table>

- Average of two assays of culture filtrate pooled from three flasks per treatment. The estimate of variance for the error component for the duplicate readings (i.e., measurement error) is 0.000504 absorbance (A) unit.
- *Values are adjusted for differences in the final filtrate volume based on mycelial weight. The relationship between filtrate volume (ml) and mycelial weight (mg) as determined in another experiment at 28 days for isolate T-154 is linear in the range of final mycelial weights encountered; MS (error) = 0.26135; 12 observations; R^2 = (−0.971)^4. The ratio of the half-width of the 95% confidence interval expressed as a percentage of the predicted mean values for weight and volume is ±5.3% of the numerical values for total A per minute per gram of mycelial dry weight per flask.

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peroxidase (type II, Sigma) were separated by polyacrylamide gel electrophoresis at 4 C and 3 ma per column in a tris(hydroxymethyl)amino methane-glycine system (1, 10). Peroxidase isoenzymes were detected with a 0.01 M benzo- dine-guaiacol mixture (13) or 0.01 M o-dianisidine as co-substrates. Soluble proteins were stained with Coomassie blue. Four horseradish isoenzymes (E_r < 0.35) were detected. However, in solutions of equal peroxidase activity (21.8 A/min/ml), P. weirii ATCC 22570 produced three isozymes with different E_r values (0.48, 0.58, and 0.72). Five bands were detected (E_r 0.19, 0.37, 0.47, and 0.91) with Coomassie blue.

Wood generally caused an increase in the concentration of peroxidase in culture filtrates. It also increased mycelial production and thus decreased the relative peroxidase production per gram of mycelium (Table 1). Malt extract may partly substitute for lignin, which aug- ments the formation of peroxidase in a chemi- cally defined medium (5), since malt also in- duces synthesis of polyphenol oxidase (2). The fluctuation in peroxidase activity between sampling dates for some isolates suggests a periodicity noted for this enzyme in other wood-rotting fungi (7, 9). These data show that P. weirii produces significant amounts of a peroxidase different from horseradish peroxi- dase and hence may be of value for com- mercial purposes.

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LITERATURE CITED