Xenobiotic induction of esterases in cultures of the yeast-like symbiont from the cigarette beetle

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Abstract

By using the 1-naphthyl acetate assay system, we found that xenobiotics can induce increased esterase activity in cultures of the yeast-like symbiont, Symbiotaphrina kochii Jurzitza ex. W. Gams and v. Arx, of the cigarette beetle, Lasioderma serricorne L.. The activities expressed as percent of solvent control are as follows: flavone, 184.2%; griseofulvin, 115.8%; cis(-)-β-pinene, 111.4%; and malathion, 114.0%. Gel electrophoresis showed new enzymes are induced by three of the compounds tested. Thus, the yeast responds in a manner consistent with detoxification enzyme regulation common in insects and microorganisms.

Introduction

Both microbial-insect and microbial-plant interactions can modify the plant-insect relationship. The ecological and evolutionary implications may involve broadening or narrowing in host range (Jones, 1984). One major mechanism by which insects avoid adverse effects of defensive plant allelochemicals is the use of several enzymes that are capable of detoxification. These enzymes can detoxify (or sometimes activate) the chemicals into one or more metabolites which may be utilized or eliminated (Ahmad et al., 1986). The allelochemicals can also induce these enzymes enabling the insect to be more tolerant to allelochemicals and related xenobiotics (Yu, 1986). In a similar manner, microbes play an important role in detoxification of pesticides (Bollag, 1982) that includes induction of detoxifying enzyme as well. While these xenobiotics may not be toxic to microorganisms per se, metabolic process of microorganisms, which may involve mineralization or cometabolism (Bollag, 1982) ultimately result in detoxification as far as higher organism are concerned (Fewson, 1988).

The cigarette beetle, Lasioderma serricorne F., can feed on a variety of hosts, e.g. tobacco, straw, many seeds and pepper (Metcalf et al., 1962). Therefore, this insect must be able to cope with a variety of allelochemicals. Recent research into the L. serricorne-yeast symbiont relationship suggests that symbiont-mediated detoxification in insect herbivores is important (Dowd, 1988a). The apparent role of the L. serricorne symbiont in detoxification has been demonstrated through examination of its hydrolytic detoxifying enzymes (Dowd, 1988b). The symbiont can also be conveniently cultured apart from the host (e.g. van der Walt, 1961). We therefore decided to test cultures of the symbiont for their response to...
xenobiotics. If the symbionts are involved in detoxification, they should respond to xenobiotics by increasing levels of detoxifying enzymes (such as esterases), in a manner analogous to studies with insect tissue.

**Materials and methods**

**Chemicals:** Sodium dodecyl sulfate (SDS), L-naphthyl acetate, N,N,N,N-tetramethyl ethylenediamine, N,N-methylene-bis-acrylamide, ammonium persulfate, nicotine, phenol, indole-3-carbinol-hydrate, griseofulvin, cis(-)-B-pinene, fast blue BB salt, and dextrose were all obtained from Sigma Chemical Co. The sources of the rest of the chemicals were as follows: Bacto agar, Difco; flavone, Aldrich Chemical Co.; malathion, Chem Service; acrylamide, BioRad; and glycine, Research Organics Inc. All other chemicals were reagent grade.

**Isolation of symbionts**

The symbiont cultures were isolated by standard techniques (Van der Walt, 1961). Fourth-instar *L. serricorne* larvae were surface sterilized in ethanol for 1 min and guts were dissected under sterile conditions. Mycetome tissues (which contain the symbiont yeast) were removed from the gut, rinsed with two changes of sterile water, and crushed onto malt extract agar. Morphology and light-induced production of pigments by the pure cultured organism was highly similar to prior reports (e.g. van der Walt, 1961; Jurzitza 1979) indicating it was the symbiont *Symbiotaphrina kochii* Jurzitza ex W. Gams and v. Arx. The yeast was maintained on potato dextrose agar (PDA) and transferred monthly. Yeast cultures used for the enzyme assays were about four-weeks old.

**Preparation of media**

All glassware was dry sterilized at 180 °C for 1.5 hours. Chemicals (20 mg) were weighed under sterile conditions, added to sterile tubes (10 × 75 mm), and dissolved in 500 μl ethanol. The solutions were added to 20 ml of liquid sterile PDA in 25 × 150 mm tubes, blended with a vortex mixer, and dispensed into 9 cm petri plates. When they cooled to room temperature, they were streak inoculated with ca. 1/4 loopful of 1–2 week old symbiont cultures.

**Esterase assay**

**Sample preparation**

Approximately two centimeters of 6 mm wide colony growth was scraped from each of the plates, and suspended into 2 ml of 0.1 M phosphate buffer, pH 7.4. Then, 0.25 ml of this suspension was diluted 1:10 with the same buffer. Absorbance at 800 nm was determined and the concentration of symbionts adjusted to about 1 AU (absorbance unit) for the enzyme assays. The esterase assay was based on a previously reported method (Dowd & Sparks, 1984). Briefly, 100 μl of yeast suspension were incubated with 2.5 × 10⁻⁴ M L-naphthyl acetate in 0.1 M, pH 7.4 phosphate buffer at 35 °C for 20 min. The reaction was quenched by addition of a SDS and fast blue BB salt solution. The diazo-L-naphthol hydrolysis product was quantitated at 600 nm with a standard curve.

**Gel electrophoresis**

To detect whether there is a qualitative change in the esterase enzymes, one ml of concentrated suspension (5 AU) was used for making a cell-free yeast extract.

The yeast was broken (ruptured) by shaking vigorously with 0.5-mm glass beads (rinsed before use) for three minutes in a minibeader (Biospec Products). The resultant above beads supernatant was centrifuged at 1,000 × g to sediment the remaining intact yeast cells, and cell-wall fragments. Ten μl of the supernatant was applied to precast wells of a 7.5% polyacrylamide gel, which was run at 35 ma constant amperage until
tracking dye (bromophenol blue) reached the opposite side of the gel, and assayed for l-naphthyl acetate esterase activity according to previously published methods (Dowd & Sparks, 1986). All assays were performed in duplicate on at least two occasions.

**Results and discussion**

The cultured yeast grew relatively well (colony width was greater than 6 mm after 4 weeks) on media containing no xenobiotics, the solvent control, flavone, griseofulvin, malathion and β-pinene, while the growth of yeast on plates containing nicotine, phenol, and 3-indole-carbinol-hydrate was rather poor (less than 2 mm); the latter three cultures were not used in the esterase assays (quantity of material not sufficient). The l-naphthyl acetate hydrolyase activity was increased by the solvent, so activity was expressed as percentage of solvent control (Table 1). Flavone was the most potent inducer while griseofulvin, malathion, and β-pinene were less effective.

From gel electrophoresis studies, no changes in the number of esterase enzymes in the cell-free extract as induced by flavone were noted (only one band—Fig. I). An additional esterase was seen with griseofulvin, malathion and β-pinene, since there were two bands present; Rf values were 0.32 and 0.36. However, the intensity of the bands did not appear to be directly proportional to the spectrophotometric enzyme assay results relative to controls. These disparities may be due to differences in the enzyme composition between the whole yeast and cell-free enzyme preparations.

Increases in enzyme activity stimulated by xenobiotics are often considered to be due to enzyme induction (Yu, 1986). Induction of esterase activity in fungi has been obtained with flavonoids. For example, in *Aspergillus flavus*, rutin and rubinin increased esterase activity by 100% and 81%, respectively; some other flavonols were of lesser activity, or had no effect (Child et al., 1963). Three species of yeast also metabolized flavonol glycosides by the same pathway as reported for *Aspergillus* spp. (Westlake & Spencer, 1965). Induction of l-naphthyl acetate esterase activity in fall armyworm, *Spodoptera frugiperda* (J. E. Smith), by plant allelochemicals was slight; flavone caused 180% increase on the esterase activity in the midgut homogenate (Yu, 1983; Yu & Hsu, 1985).

There were no increases in midgut esterase activity when tobacco budworm, *Heliothis virescens* (F.), larvae were fed dietary (+)-x-pinene, 5-caryophyllene, gossypol, umbelliferone and scopoletin (Brattsten, 1987). Similarly, feeding adults and larvae of the boll weevil (*Anthonomus grandis grandis* Boheman) these same xenobiotics had no effect on soluble esterase activity, except for a moderate increase in microsomal esterase in the larvae when fed 0.2% gossypol diet (Brattsten, 1987). Thus, similar to some findings reported for hydrolytic enzymes from fungi and insects, we found xenobiotics can induce esterase activity with the yeast-like symbionts of *L. serricorne*.

An esterase is an enzyme that catalyzes the hydrolysis of an ester bond without any requirement for high-energy cofactors (IUB, 1984). Many of these esterases have broad substrate specificities, and may be important in the metabolism of allelochemicals (Ahmad et al., 1986). The assay of l-naphthyl acetate is an indicator of general esterase activity, the presence of which may be the reason that an insect is able to detoxify

### Table 1. Effect of xenobiotics on esterase activity in symbiont cultures from *Lasioderma serricorne* L.

<table>
<thead>
<tr>
<th>Xenobiotics (0.1%)</th>
<th>Specific activity (nmole min AU)</th>
<th>SC *</th>
<th>SC **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no solvent)</td>
<td>0.850 ± 0.015</td>
<td>89.5*</td>
<td></td>
</tr>
<tr>
<td>Solvent control (SC)</td>
<td>0.950 ± 0.013</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Flavone</td>
<td>1.750 ± 0.038</td>
<td>184.2**</td>
<td></td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>1.100 ± 0.048</td>
<td>115.8*</td>
<td></td>
</tr>
<tr>
<td>cis-β-pinene</td>
<td>1.058 ± 0.025</td>
<td>111.4*</td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>1.083 ± 0.042</td>
<td>114.0*</td>
<td></td>
</tr>
</tbody>
</table>

* Value significantly different (*p* < 0.05) from the solvent control (SC) by analysis of variance.

** Value highly significantly different (*p* < 0.01) from the solvent control (SC) by analysis of variance.
allelochemicals that have ester linkages (Ahmad et al., 1986). For example, tannin is detoxified to gallic acid by hydrolases in certain tree-feeding locusts (Bernays, 1978). The product is utilized in the cross-linking of cuticular proteins (Bernays & Woodhead, 1982). There are also several examples where esterases are involved in malathion detoxification in insects (Oppenoorth & Welling, 1976). Thus, the induction of l-naphthyl-acetate esterase activity in the _L. serricorne_ symbionts is likely to be a detoxification-associated response.

We found no oxidative or glutathione S-transferase activities in the symbiont cultures when previously published methods were used (Rose & Brindley, 1985; Dowd et al., 1986, respectively). However, prior comparable work suggests these enzymes may also be induced. For example, compared to esterases, microsomal epoxidase and glutathione S-transferase can be induced by nearly 5-fold to 26-fold, respectively, by the same allelochemicals in _S. frugiperda_ (Yu & Hsu, 1985). Induction of alkane hydroxylase and cytochrome P-450 content in growing yeast, _Candida tropicalis_, exposed to appropriate alkanes, alkenes, and alcohols has also been reported (Gilewicz et al., 1979).

We conclude that induction of esterase enzymes in cultures of _L. serricorne_ symbionts does occur. Although the response may differ in the host, the ability of the symbiont to produce more of a detoxifying enzyme in the presence of a variety of xenobiotics, coupled with past examination of the detoxifying capabilities, of the symbionts that show significant decreases in enzyme activities and increased susceptibility to toxins in the absence of symbionts (Dowd,
1988a, b) further demonstrate that the symbiont is likely to be an important component of the detoxification mechanisms of their insect host.

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other forms or similar products not mentioned.

Résumé

Induction xenobiotique d'estérases dans des cultures de symbiontes du type levure de Lasioderma serricorne

En utilisant une méthode de dosage avec le l-naphtyle-acétate, nous avons trouvé que des substances xenobiotiques peuvent induire une augmentation de l'activité estérase dans des cultures du symbionte de type levure, Symbiotaphrina kochii Jurzitza, ex W. Gams & v. Arx, de L. serricorne. Le solvant ayant servi de réaction a constitué la référence à partir de laquelle ont été calculés les pourcentages obtenus: flavone 184,2 %; griséofluvène, 115,8 %; cis(-)-β-pinène, 111,4 %; malathion, 114,0 %. L'électrophorèse sur gel de polyacrylamine a montré que les nouvelles enzymes sont induites par 3 des substances examinées. Ainsi, la levure réagit d'une façon compatible avec la régulation enzymatique de la détoxication commune aux insectes et aux microorganismes.

References


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