Fungal Metabolism of \( n \)-Alkylbenzenes

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Received 23 July 1985/Accepted 28 October 1985

Isolates of Paecilomyces, Verticillium, Beauveria, and Penicillium species were tested for ability to metabolize a variety of \( n \)-alkylbenzenes. Minimum side chain lengths were required for metabolism of these substrates.

These were \( C_4 \) for the Paecilomyces sp., \( C_6 \) for the Verticillium sp., and \( C_7 \) for the other two isolates. Growth on dodecylbenzene yielded benzoic and phenylacetic acids as transient intermediates, and these acids supported growth of the isolates.

Numerous studies have reported the ability of fungi to metabolize a variety of aromatic hydrocarbons (3, 5, 6, 8, 10, 17). Typically, metabolites are hydroxylated derivatives of the aromatic substrates. Transformations of alkyl-substituted aromatic compounds, such as methyl-naphthalenes (7) and 7-methylbenzanthracene (4), also have been reported. No evidence of ring cleavage was reported in any of these studies.

In this study, four \( n \)-alkane-degrading fungi isolated from the marine environment (11) were grown in the presence of \( n \)-alkylbenzenes to determine the effect of side chain length (\( C_1 \) to \( C_{12} \)) on substrate utilization. Metabolites of dodecylbenzene (DB) were identified in batch cultures.

Isolates of Paecilomyces, Beauveria, Penicillium, and Verticillium species were streaked onto six slants of basal marine agar containing N and P (11) and grown for 7 days at 28°C. Sterile 0.1-ml portions of hexylbenzene, octylbenzene, nonylbenzene, decylbenzene, and DB were then added to individual cultures. The sixth replicate of each culture received 0.3 ml of 970 mM sodium benzoate. Equal volumes of each substrate were added to sterile slants to serve as controls. After a further 28 days of incubation, each slant was extracted with 5 ml of \( n \)-pentane, and the amount of substrate remaining was determined by gas chromatography (GC).

Growth of these isolates on volatile substrates (benzene, toluene, ethylbenzene, butylbenzene, and hexylbenzene) was tested by using a well flask method (13). Dry weights of mycelium (2) were determined after 30 days of incubation.

The data in Table 1 summarize the percentage of substrate lost from slants due to fungal metabolism. The well flask experiments showed that the vapors from benzene and toluene inhibited the growth of all four isolates, and only the Paecilomyces sp. isolate could grow on butylbenzene and hexylbenzene. These results clearly show that there is a minimum \( n \)-alkyl side chain length required for the growth of these fungi. The Paecilomyces sp. will grow if a \( C_4 \) side chain is present, the Verticillium sp. requires a \( C_6 \) side chain, and the Beauveria sp. and Penicillium sp. require a \( C_9 \) side chain.

The metabolites of DB degradation were identified by growing the Paecilomyces sp. culture in 200 ml of a liquid basal marine medium (BMM) (11) containing 2 ml of NP solution (12) and 0.5 ml of sterile DB. This culture was incubated without shaking, and after 8 days it was acidified with 2 ml of 6 M HCl. The fungal mat was recovered by filtration (Whatman no. 1 paper) and washed with 25 ml of methylene chloride. The filtrate was extracted with methy-

### Table 1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Paecilomyces</th>
<th>Verticillium</th>
<th>Beauveria</th>
<th>Penicillium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Toluene</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Butylbenzene</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Hexylbenzene</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

* Corresponding author.
TABLE 1. Extent of substrate metabolism after fungal cultures had been incubated for 28 days in the presence of benzene derivatives

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Benzoic acid</th>
<th>Hexyl-benzene</th>
<th>Octyl-benzene</th>
<th>Nonyl-benzene</th>
<th>Decyl-benzene</th>
<th>DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium</td>
<td>100</td>
<td>0</td>
<td>26</td>
<td>54</td>
<td>48</td>
<td>99</td>
</tr>
<tr>
<td>Beauveria</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>90</td>
<td>75</td>
<td>99</td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>100</td>
<td>55</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Verticillium</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
</tbody>
</table>

* Amounts of substrate recovered from fungal cultures compared with amounts recovered from sterile controls.

The aromatic acids were not as fast as the rate of metabolism of the n-alkyl side chain.

During the growth of the Penicillium sp. on DB (Fig. 1b), benzoic, phenylacetic, phenylpropionic, and cinnamic acids were detected in the medium. However, these did not accumulate in the same manner as was observed with the Paecilomyces sp. isolate (Fig. 1a), indicating that the aromatic moiety was metabolized at a rate similar to that of the n-alkyl side chain.

Each of the four isolates was tested for its ability to grow on benzoic and phenylacetic acids. Volumes of 30 ml of BMM plus 0.3 ml of NP solution were supplemented with 200 mg of each acid (as their sodium salts). Control flasks containing only BMM and NP solution were prepared for each isolate. Equal volumes of homogenized inocula were added to each flask and these were incubated for 14 days, after which dry weight of the mycelium was determined (Table 2). Based on the Student t test (P < 0.05), the cell yields of all four isolates were greater in media supplemented with benzoic or phenylacetic acid than in the control medium.

FIG. 1. Loss of DB and production of aromatic acids in liquid cultures of (a) Paecilomyces sp. and (b) Penicillium sp.
dium, thus directly confirming that the four fungi metabolize these acids. Similar studies with _N. salmonicolor_ (16) growing on DB have also demonstrated the transient appearance of aromatic acids. Our observations are in contrast to those of Davis and Raymond (9), who found that phenylacetic acid accumulated in cultures of several _Nocardia_ sp. strains grown on DB. In summary, this study has shown that the ability of four fungal isolates to metabolize _n_-alkylbenzenes is dependent upon the length of the alkyl portion. If the side chain is long enough, the _n_-alkyl portion is first attacked, yielding benzoic and phenylacetic acids as intermediates. These aromatic acids then also serve as growth substrates.

We acknowledge the fine technical assistance of C. Anders and L. Jackson and thank D. Morgan of the Chemistry Department for the GC-MS analyses.

This work was supported by Natural Sciences and Engineering Research Council of Canada operating grant A-3687.

**LITERATURE CITED**


