NOTES

Initial Reactions Involved in the Dissimilation of Mandelate by
*Rhodotorula graminis*

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Received 11 June 1984/Accepted 21 August 1984

**Rhodotorula graminis** utilized DL-mandelate, L(+)-mandelate, and D(-)-mandelate as sole sources of carbon and energy. Growth on these aromatic substrates resulted in the induction of an NAD-dependent D(-)-mandelate dehydrogenase and a dye-linked L(+)-mandelate dehydrogenase, each catalyzing the stereospecific conversion of its respective enantiomer of mandelate to benzoylformate. Benzoylformate was oxidized to benzaldehyde, which was dehydrogenated to benzoate by an NAD-dependent benzaldehyde dehydrogenase. Benzoate was further metabolized through p-hydroxybenzoate and the protocatechuic branch of the β-ketoacidipate pathway.

Evidence for a dissimilatory pathway for mandelate utilization by yeasts was recently reported by Durham et al. (1). Biochemical genetic studies revealed that *Rhodotorula graminis* metabolized DL-mandelate through benzoate and the protocatechuate branch of the β-ketoacidipate pathway. Thus, mandelate catabolism in *R. graminis* appears to be analogous to that reported for the filamentous fungus *Aspergillus niger* (11). Conversely, bacteria, particularly *Pseudomonas putida*, metabolize mandelate through catechol rather than protocatechuic (5–8, 12, 14). In addition, bacteria utilize disparate enzymatic reactions that are not observed in *A. niger* for the initial oxidation of mandelate. This report describes the enzyme activities involved in the oxidation of mandelate to benzoate in *R. graminis*. The results indicate that the mandelate dissimilatory enzymes in *R. graminis* more closely resemble those described in *A. niger* than the mandelate enzymes reported for *P. putida* (8). Unlike *P. putida*, *R. graminis* expresses separable, stereospecific enzymes for the dehydrogenation of D(-)-mandelate and L(+)-mandelate. Some properties of these enzymes are described in this communication.

Growth and maintenance of *R. graminis* KGX39 as well as the preparation of crude extracts and the fractionation of extracts via ultracentrifugation were performed as previously described (1, 3). Benzoylformate decarboxylase (EC 4.1.1.7) (8), benzoate-4-hydroxylase (1), p-hydroxybenzoate-3-hydroxylase (EC 1.14.13.2) (1), and protocatechuic 3,4-dioxygenase (EC 1.13.11.3) (4) were assayed by published procedures. L(+)-Mandelate dehydrogenase was assayed as described by Hegeman (8) unless otherwise noted. D(-)-Mandelate dehydrogenase was assayed spectrophotometrically by measuring the reduction of NAD at 340 nm. The reaction mixtures contained in a final volume of 1 ml: 200 μmol of Tris-hydrochloride (pH 8.0), 0.5 μmol of NAD, 0.5 μmol of D(-)-mandelate, and extract. Protein was determined by the procedure of Lowry et al. (13).

The ability of *R. graminis* to utilize isomers of mandelate and benzoylformate was examined by performing diauxic growth experiments in basal salts medium (3) containing 2 mM glucose and 10 mM aromatic substrate. Glucose-grown inocula of *R. graminis* preferentially grew on glucose, and after a 1.5- to 2-h lag period, cells utilized the aromatic substrate. *R. graminis* grew equally well on L(+)-mandelate, D(-)-mandelate, and DL-mandelate. In addition DL-p-hydroxymandelate and benzoylformate supported growth. Hegeman et al. (10) reported that an unidentified yeast utilized the L(+) isomer of mandelate, whereas Jamaluddin et al. (11)

![Diagram](http://jb.asm.org/Downloaded from http://jb.asm.org on November 6, 2017 by guest)
TABLE 1. Specific activities of mandelate dissimilatory enzymes in *R.* *graminis*<sup>a</sup>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sp act (nmol/min per mg of protein) on growth substrate:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DL-Mandelate</td>
</tr>
<tr>
<td>D(-)-Mandelate dehydrogenase</td>
<td>385</td>
</tr>
<tr>
<td>L(+)Mandelate dehydrogenase</td>
<td>147</td>
</tr>
<tr>
<td>Benzyloformate decarboxylase</td>
<td>203</td>
</tr>
<tr>
<td>Benzaldehyde dehydrogenase</td>
<td>153</td>
</tr>
<tr>
<td>Benzoate-4-hydroxylase</td>
<td>20</td>
</tr>
<tr>
<td>p-Hydroxybenzoate-3-hydroxylase</td>
<td>64</td>
</tr>
<tr>
<td>Protocatechuic acid 3,4-dioxygenase</td>
<td>201</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cells were grown on basal salts medium containing 20 mM substrate. Extracts were prepared as described in the text.

demonstrated that *A.* *niger* utilizes DL-mandelate; no data are available for the utilization of the individual D(-) or L(+) isomers were reported for *A.* *niger* (11).

Specific activities of mandelate dissimilatory enzymes were determined after growth of *R.* *graminis* on aromatic substrates (Table 1). Crude extracts prepared from glucose-grown cells were assayed to determine basal enzyme levels. Extracts from DL-mandelate-grown cells contained induced levels of D(-)mandelate and L(+)-mandelate dehydrogenases, benzoylformate decarboxylase, and benzaldehyde dehydrogenase as well as the benzoate dissimilatory enzymes previously described (1; Table 1). All of the enzymes, perhaps with the exception of L(+)-mandelate dehydrogenase (see below), were soluble proteins and, in most cases, were not detected in extracts prepared from glucose-grown cells. Benzaldehyde dehydrogenase was detected in high basal levels (Table 1); unlike *P.* *putida* (8) and *A.* *niger* (11), which contain both NAD- and NADP-specific benzaldehyde dehydrogenases, *R.* *graminis* contains only one form of the enzyme which was NAD-dependent. Growth of *R.* *graminis* on D(-)- or L(+)-mandelate resulted in induced levels of mandelate dissimilatory enzymes comparable to those observed after growth on DL-mandelate (Table 1). Extracts from benzoylformate-grown cells contained low levels of L(+)- and D(-)-mandelate dehydrogenases (Table 1); it is unclear whether benzoylformate can serve as an inducer for these enzymes or whether the substrate is being slowly reduced within the cell, forming isomers of mandelate which induce these enzymes. Experiments with mutants of *P.* *putida* revealed that benzoylformate can act as an inducer without being reduced to mandelate (9).

The initial reaction for mandelate metabolism in *P.* *putida* differs from that of *A.* *niger* and *R.* *graminis*. For example, in *P.* *putida* a soluble racemase mediates the conversion of D(-)-mandelate to L(+)-mandelate, which is then dehydrogenated to benzoylformate by a membrane-associated, dye-linked L(+)-mandelate dehydrogenase (5, 6, 10, 14; Fig. 1). In contrast, *A.* *niger* contains a membrane-associated D(-)-mandelate dehydrogenase and a soluble, dye-linked L(+)-mandelate dehydrogenase which can form benzoylformate from their respective isomers (11). Results from this investigation indicate that *R.* *graminis* is similar to *A.* *niger* in that it possesses both L(+)- and D(-)-mandelate dehydrogenases (Table 1) and not a racemase. However, the D(-)-mandelate dehydrogenase from *R.* *graminis* is a soluble dehydrogenase which is NAD-dependent; no activity was detected with NADP. Moreover, dye-linked L(+)-mandelate dehydrogenase activity was observed in the soluble as well as the membrane fraction of the cell (data not shown). It is probable that this enzyme is membrane associated but is readily solubilized during cell extract preparation.

Electrophorograms (2) of soluble extracts from DL-mandelate-grown cells indicated that the L(+)- and D(-)-mandelate dehydrogenases are separate, electrophoretically distinct proteins with relative mobilities of 0.15 and 0.39, respectively. Additional properties of the L(+)- and D(-)-mandelate dehydrogenases are summarized in Table 2. Besides exhibiting a strict stereospecificity for their respective substrate, different electron acceptor specificities, and subcellular location, the enzymes differed in pH optima and thermal stability. D(-)-Mandelate dehydrogenase and L(+)-mandelate dehydrogenase had optimal activities at pH 9.0 and 7.0, respectively, and the half-life of the respective dehydrogenases at 52°C was 1.5 and 45 min (Table 2). These results favor the conclusion that *R.* *graminis* contains separate, stereospecific mandelate dehydrogenases.

The results from this study demonstrate for the first time a dissimilatory pathway for mandelate utilization in yeasts. The mandelate pathway in *R.* *graminis* is similar to that described for *A.* *niger* with the exception of noted differ-

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<sup>a</sup> Comparisons were made with the soluble fraction obtained from ultracentrifugation of extracts of DL-mandelate-grown cells.

<sup>b</sup> At 52°C.

<sup>c</sup> Activity was stimulated twofold by the addition of 1.0 mM phenazine methosulfate (PMS).
ences in the enzymes involved. The initial reactions involved in the metabolism of \( L^+ \) and \( D^- \) isomers of mandelate are catalyzed by stereospecific dehydrogenases which convert mandelate to benzoylformate, which in turn is metabolized through benzoate and the protocatechuate pathway (Fig. 1).

LITERATURE CITED