Localization of Arylsulfatase in *Pseudomonas* C$_{12}$B

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Arylsulfatase was released almost completely from intact cells of *Pseudomonas* C$_{12}$B after osmotic shock or after treatment with lysozyme. These results suggest that the enzyme is cell wall associated in this soil isolate.

Arylsulfatase (arylsulfate sulfohydrolase, EC 3.1.6.1) catalyzes the release of $\text{SO}_4^{2-}$ from sulfate esters of simple phenols. In view of the widespread occurrence of arylsulfate esters in nature, microorganisms synthesizing this enzyme are considered by many to be important for sulfate ester mineralization in soils that contain high levels of sulfur in the form of ester sulfate (7, 16). Tabatabai and Bremner (13, 14, 15) were the first to demonstrate that Iowa soils that are rich in ester sulfate (up to 93% of total soil S) also exhibit appreciable arylsulfatase activity. Further studies (1, 2, 8, 9) have confirmed these findings for other soils, and there is evidence (13) that much of the soil arylsulfatase activity is associated with microorganisms. To assess the involvement of intact cells in sulfate ester mineralization, it is necessary to know the cellular location of the responsible enzymes. Despite indirect evidence suggesting an exocyttoplasmic location for arylsulfatase, there is no direct evidence available for any one bacterium (4). This study was undertaken to determine the location of the enzyme in a soil bacterium isolated and identified by Payne and Feisal (11, 12) as a *Pseudomonas* species.

Bacteria, derepressed for arylsulfatase formation (6), were subjected to osmotic shock as follows: cells (0.2 g, wet weight) were suspended in 25 ml of 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 8.0, containing 20% (wt/vol) sucrose and varying concentrations of sodium ethylenediaminetetraacetate (EDTA). Cells were harvested by centrifugation after shaking at 30°C for 10 min, and the cell pellet was suspended in 25 ml of water containing varying concentrations of MgCl$_2$. This suspension was agitated at 30°C for 10 min, followed by centrifugation and cell rupture. Cell extracts, wash fluids, shock fluids, and culture supernatants were dialyzed and assayed directly as previously described (5, 6).

Arylsulfatase activity of shock fluids was dependent upon the EDTA concentration of the preliminary wash solution (Fig. 1A), as well as upon the MgCl$_2$ content of the solution subsequently used to shock the cells (Fig. 1B). Maximum enzyme release occurred when cells were washed in sucrose and Tris containing 1 mM EDTA and then shocked in water or 0.1 mM MgCl$_2$. Very little enzyme (3.8%) was released during the preliminary wash containing 1 mM EDTA, and cells subsequently shocked in 0.1 mM MgCl$_2$ released 94% of their original arylsulfatase content (Table 1). As determined by growth in nutrient broth at 30°C (see reference 5 for procedure), the viability of shocked cells was identical to that of untreated cells. This result agrees with previous work with this isolate (5) and with earlier work (10) which demonstrated that inclusion of MgCl$_2$ in shock fluids enhanced viability of members of the *Enterobacteriaceae* after shock. Resuspension of washed cells in 25 ml of 10 mM Tris-hydrochloride buffer, pH 8.0, containing 20% sucrose and lysozyme (0.5 mg/ml) resulted in a 91%
release of arylsulfatase after incubation at 30°C for 10 min (Table 2).

Collectively, these results suggest that arylsulfatase is cell wall associated in Pseudomonas C₁₂B. Moreover, the almost total release of the enzyme after osmotic shock indicates a periplasmic rather than a surface location. This possibility is supported further by our failure to detect significant activity in wash fluids or in culture supernatants (Tables 1 and 2). Arylsulfatase thus differs in its cellular location from the alkylsulfatases, which appear to be distributed throughout the cell wall of this isolate (5).

LITERATURE CITED

5. Fitzgerald, J. W., and W. W. Leslie. 1975. Loss of primary alkylsulfatase and secondary alkylsulfatases (S-1 and S-2) from Pseudomonas C₁₂B: effect of cul-

TABLE 1. Release of arylsulfatase from Pseudomonas C₁₂B by osmotic shock a

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (EU)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>0.19</td>
</tr>
<tr>
<td>Untreated cells</td>
<td>5.60</td>
</tr>
<tr>
<td>Wash fluid</td>
<td>0.21</td>
</tr>
<tr>
<td>Shock fluid</td>
<td>5.24</td>
</tr>
<tr>
<td>Treated cells</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Cells washed with 10 mM Tris-hydrochloride buffer, pH 8.0, containing 20% sucrose-1 mM EDTA and then shocked in 0.1 mM MgCl₂. See text for further details.

b One enzyme unit (EU) is the amount of enzyme necessary to liberate 1 μmol of p-nitrocatechol from 10 mM p-nitrocatechol sulfate in 1 min at pH 7.5 and 30°C.

TABLE 2. Release of arylsulfatase from Pseudomonas C₁₂B after treatment with lysozyme a

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (EU)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>0.27</td>
</tr>
<tr>
<td>Untreated cells</td>
<td>5.93</td>
</tr>
<tr>
<td>Wash fluid</td>
<td>0.30</td>
</tr>
<tr>
<td>Lysozyme wash</td>
<td>5.41</td>
</tr>
<tr>
<td>Treated cells</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Cells washed initially with 10 mM Tris-hydrochloride buffer, pH 8.0, containing 20% sucrose-1 mM EDTA (wash fluid) and then washed with the same solvent containing lysozyme (0.5 mg/ml) but lacking EDTA. See text for further details.

b See Table 1.