Phospholipid Biosynthesis and Solvent Tolerance in Pseudomonas putida Strains

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The role of the cell envelope in the solvent tolerance mechanisms of Pseudomonas putida was investigated. The responses of a solvent-tolerant strain, P. putida Idaho, and a solvent-sensitive strain, P. putida MW1200, were examined in terms of phospholipid content and composition and of phospholipid biosynthetic rate following exposure to a nonmetabolizable solvent, o-xylene. Following o-xylene exposure, P. putida MW1200 exhibited a decrease in total phospholipid content. In contrast, P. putida Idaho demonstrated an increase in phospholipid content 1 to 6 h after exposure. Analysis of phospholipid biosynthesis showed P. putida Idaho to have a higher basal rate of phospholipid synthesis than MW1200. This rate increased significantly following exposure to xylene. Both strains showed little significant turnover of phospholipid in the absence of xylene. In the presence of xylene, both strains showed increased phospholipid turnover. The rate of turnover was significantly greater in P. putida Idaho than in P. putida MW1200. These results suggest that P. putida Idaho has a greater ability than the solvent-sensitive strain MW1200 to repair damaged membranes through efficient turnover and increased phospholipid biosynthesis.

In the past several years, many organic solvent-tolerant organisms have been discovered (1, 8, 20, 30, 35). The phenomenon of solvent tolerance is an important one, because it allows microorganisms to grow in high concentrations of organic solvents. Organisms capable of surviving these conditions have great application in bioremediation of contaminated sites and “end of the pipe” biotransformation of industrial wastes, and many have potential for use in biphasic bioconversion systems (9). Solvent-tolerant Pseudomonas strains capable of producing solvent-stable lipases and proteases have been isolated recently, further expanding the utility of these organisms (30, 31).

The mechanism for solvent tolerance is unknown, but some conclusions can be made from the current literature. The toxic effects of aromatic hydrocarbons are associated with loss of cytoplasmic membrane integrity (10, 37–39, 44). This loss of membrane integrity results in disruption of proton motive force, loss of membrane barrier functions, inhibition of membrane protein function, and subsequent cell lysis and death. Solvent tolerance does not appear to be related to degradation, as most solvent-tolerant organisms tolerate a wide variety of aromatic hydrocarbons, alkanes, and alcohols that they are incapable of degrading or transforming (8, 20, 35, 44). Most of these organisms do not harbor plasmds, indicating that the resistance factors reside on the chromosome (8, 35). Extrusion pumps similar to those described for antibiotic resistance have also been postulated as having a role in solvent tolerance (21). The 1996 study by Isken and de Bont showed that an intact proton motive force was required for solvent tolerance and suggested that this energy was harnessed by an efflux system to pump out toluene from the cell (21). Because much of the initial damage caused by straight-chain aromatic hydrocarbons occurs by disruption of phospholipid membranes (10, 22, 37–39, 48), the membrane structure of solvent-resistant strains warrants further study. In general, several mechanisms for decreasing membrane fluidity (due to temperature increases, solvents, etc.) have been demonstrated in bacteria. These include alteration of the polar head groups of phospholipids (26, 27, 41), increased relative percentage of saturated fatty acids leading to a decrease in membrane fluidity (19), and conversion of cis-unsaturated fatty acids to the trans isomer (15, 16, 45). trans-Unsaturated fatty acids are made by solvent-sensitive strains (14, 31) as well as by bacteria under starvation conditions (13), and thus their production appears to be a general stress response in some bacteria. Pseudomonas putida Idaho (8) grows in high concentrations of organic solvents and thus likely harbors some mechanism that either prevents or compensates for membrane damage.

In a previous study, it was observed that P. putida Idaho showed an increase in the amount of total membrane phospholipid fatty acid produced following exposure to xylene (33). One possible explanation for this is increased rate of membrane biosynthesis in response to exposure to xylene. An increased rate of membrane biosynthesis would be beneficial to this strain, as it would allow rapid repair of membrane damaged by exposure to xylene.

In this study, the objectives were to characterize the responses of two strains of P. putida to various concentrations of o-xylene in terms of growth and with respect to membrane adaptation in the presence and absence of monoaromatic hydrocarbons. The first goal was to determine differences in phospholipid structure and composition between P. putida Idaho and P. putida MW1200 when each strain is exposed to increasing concentrations of o-xylene. Previously observed changes in fatty acid concentration (33) were assigned to specific phospholipids. Following establishment of these differences, the second goal of this study was to determine the rates of membrane biosynthesis and phospholipid turnover in both solvent-tolerant and solvent-sensitive P. putida strains.
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MATERIALS AND METHODS

Strains and culture conditions. Two strains of *P. putida* were used. Both strains contain genes for the degradation of toluene on the chromosome (8, 40), and neither strain can degrade o-xylene. *P. putida* Idaho was first isolated by James Wolfram and Robert Rogers at Idaho National Engineering Laboratories (U.S. patent, 4,683,872, September 1989). This strain withstands aqueous solutions saturated with toluene and xylenes (8). The second strain used for these experiments is *P. putida* MW1200. This strain can tolerate a maximum of 75 ppm of o-xylene. Exposure to concentrations greater than 75 ppm causes loss of viability (33).

Unless otherwise stated, for all experiments each strain was grown in continuous culture, using a minimal salts medium with 2.7 g of sodium succinate per liter as the carbon source. The minimal salts medium was formulated as follows: 0.7 g of KH₂PO₄, 0.5 g of (NH₄)₂SO₄, 0.3 g of MgSO₄, and 1 ml of Wolfe’s minerals (3) per liter of nanopure water (pH 6.8). For the experiments using organic solvents, o-xylene was added either directly to the aqueous phase of the medium or through the feed medium of the continuous culture. The concentration of o-xylene in the aqueous phase was verified by gas chromatographic analysis.

Growth rates were previously established for each strain in the presence and absence of o-xylene (34). *P. putida* MW1200 demonstrated a doubling time of 1.4 h in both the absence and presence of 75 ppm of o-xylene. *P. putida* Idaho showed doubling times of 1.4 h in the absence of o-xylene and 1.5 h in the presence of o-xylene. This difference was not statistically significant.

Phospholipid extraction and identification of phospholipid class. Cells (100 ml) were removed from continuous culture (in the absence or presence of o-xylene) and centrifuged, the supernatant was discarded, and the cell pellet was lyophilized. The pellet was then extracted by a modified Bligh-Dyer technique (12). The organic extract containing the total lipid portion was applied to a silicic acid column. Phospholipids were eluted with methanol following chloroform and acetone washes of the column, which removed neutral lipids and glycolipids.

The specific lipid fraction obtained from silicic acid separation was further separated into phospholipid classes by one-dimensional thin-layer chromatography (TLC) (24) and developed in chloroform-methanol-water-acetic acid (85:30:8:1) (25). The organic extract containing the total lipid portion was applied to a TLC plate to isolate the individual phospholipids (24). Radioactivity was measured directly from the TLC plate by using the Bioscan Imaging Scanner system (2). Following data acquisition from the TLC plate, all radioactive spots were removed and subjected to fatty acid analysis for quantitation purposes. Specific activity was then calculated from these data. This experiment generated total radioactivity of incorporation of radiolabel into phospholipid, total radioactivity of loss from phospholipid, and incorporation values and turnover data for individual phospholipids.

Statistics. Data points shown are averages of three independent experiments, with all error bars on graphs representing 1 standard deviation. Calculations were performed with GraphPad Prism software (San Diego, Calif.). Values in tables are averages of at least three experiments and are shown as means ± 1 standard deviation. *t* tests were used to determine significant differences, using α = 0.01.

Total phospholipid biosynthesis and turnover rates were calculated by linear regression analysis. Rates of biosynthesis and turnover are the results of three independent experiments. Each data point represents the mean of three experiments. Error bars on the graphs represent 1 standard deviation. *t* tests were used to determine significant differences between the biosynthesis and turnover rates of each strain under the different growth conditions (α = 0.01).

**TABLE 1.** Mole percentages of each class of phospholipids and total phospholipid obtained from *P. putida* MW1200 and *P. putida* Idaho in the presence and absence of o-xylene

<table>
<thead>
<tr>
<th>PL class</th>
<th>MW1200</th>
<th>Idaho</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppm</td>
<td>75 ppm</td>
</tr>
<tr>
<td>PE</td>
<td>55.97</td>
<td>66.14</td>
</tr>
<tr>
<td>PG</td>
<td>28.79</td>
<td>30.74</td>
</tr>
<tr>
<td>CL</td>
<td>11.24</td>
<td>6.60</td>
</tr>
<tr>
<td>PA</td>
<td>2.40</td>
<td>1.12</td>
</tr>
<tr>
<td>PS</td>
<td>1.56</td>
<td>0.78</td>
</tr>
<tr>
<td>Total PL (nmol of phospholipid/mg [dry wt] ± 1 SD)</td>
<td>77.0 ± 13.2</td>
<td>38.4 ± 10.7</td>
</tr>
</tbody>
</table>

* PL, phospholipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PA, phosphatidic acid; PS, phosphatidylserine.

**RESULTS**

Phospholipid composition. The phospholipid composition of each strain is shown in Table 1. Initial characterization of the phospholipid composition showed that the major classes of phospholipid of both strains were phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin (diphasatidylglycerol). HPLC analysis showed the Idaho strain to have a greater percentage of phosphatidylethanolamine than *P. putida* MW1200 when grown with succinate. Phosphatidylserine and phosphatidic acid were detected in trace amounts in both strains. Following exposure to xylene, changes in phospholipid content were seen in both strains. Strain Idaho showed an overall increase in phospholipid following exposure to 75 and 200 ppm of o-xylene. Strain MW1200 showed a decrease in the amount of phospholipid following exposure to its maximum tolerable limit of xylene.
Analysis of the fatty acid composition of each phospholipid for each strain showed that specific changes in the fatty acid composition were localized to specific classes of phospholipid (Table 2). For both strains, the cis-trans isomerization occurred mainly in phosphatidylethanolamine. The increase in saturation in strain Idaho occurred mainly in phosphatidylglycerol. Strain MW1200 showed a similar but smaller increase in saturation associated with phosphatidylglycerol (not shown). Phosphatic acid showed small changes in both saturation and levels of cis-unsatuated fatty acids. Cardiolipin did not show much change in fatty acid composition when it was grown under conditions of xylene saturation.

Previous analysis of the lipid A from the lipopolysaccharide (LPS) of each strain showed no compositional change for both strains grown in the presence and absence of o-xylene, although strain Idaho showed increased total LPS following xylene exposure (33, 34).

**Table 2. Changes in fatty acid composition of individual phospholipids of P. putida Idaho following exposure to o-xylene**

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>PE</th>
<th>PG</th>
<th>CL</th>
<th>PA</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>Xyl</td>
<td>+Xyl</td>
<td>Xyl</td>
<td>+Xyl</td>
<td>Xyl</td>
</tr>
<tr>
<td>cis unsaturated</td>
<td>59±12</td>
<td>60±12</td>
<td>54±9</td>
<td>88±13</td>
<td>97±8</td>
</tr>
<tr>
<td>trans unsaturated</td>
<td>35±6</td>
<td>17±4</td>
<td>46±7</td>
<td>9±3</td>
<td>3±0.6</td>
</tr>
</tbody>
</table>
| Mol% (mean ± 1 SD [n = 3]) | PE, phosphatidylyethanolamine; PG, phosphatidylglycerol; CL, cardiolipin; PA, phosphatidic acid; PS, phosphatidylserine.

Following exposure to 75 ppm of xylene, the amount of phospholipid synthesized was less than that observed for growth in the absence of the solvent. In the presence of 200 ppm of xylene, loss of phospholipid was noted over time, indicating cell death. This loss was small in the initial 2 h of exposure but accelerated soon after the 2-h time point.

**Phospholipid biosynthesis and turnover.** Cell numbers at the start of the experiment were 3.1 (±0.8) × 10^7 CFU/ml for P. putida MW1200 and 4.1 (±1.2) × 10^7 CFU/ml for P. putida Idaho. Ending cell numbers were 6.3 (±1.1) × 10^7 and 5.9 (±1.4) × 10^7 CFU/ml, respectively, indicating a small increase in population over the course of the experiment. Lipid biosynthesis rates were normalized to the increase in cell number over time. The azide-killed cells showed minimal uptake of radiolabel, with incorporation of an average of 0.12 cpm mmol⁻¹ min⁻¹. This value was subtracted from the rate calcu-
lated for each experimental strain. Incorporation of label into phospholipid was monitored over time (average of three experiments) and is expressed as specific activity in Fig. 2. Strain MW1200 showed incorporation of $^{32}$P into phospholipid at an average rate of 207.3 ($\pm 21.04$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.907$). In the presence of 75 ppm of $o$-xylene, this rate increased to 273.6 ($\pm 17.00$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.963$). Strain Idaho showed a higher rate of incorporation. In the absence of solvent, strain Idaho exhibited an average incorporation rate of 532.7 ($\pm 23.16$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.981$). In the presence of 75 ppm of $o$-xylene, this rate increased to 852.0 ($\pm 31.47$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.987$). The incorporation rate for $P. putida$ Idaho in the presence of 200 ppm of xylene was 882.6 ($\pm 29.23$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.989$). All incorporation rates were significantly linear.

$t$ tests (Table 3) run on the following data showed the following results: (i) for strain MW1200, there was no significant difference between the rates of incorporation of radiolabel into phospholipid in cells grown in the presence and absence of $o$-xylene; (ii) there was no significant difference between the rates of incorporation of $P. putida$ Idaho grown in the presence of 75 ppm of $o$-xylene and 200 ppm of $o$-xylene; (iii) there was a significant difference between the rates of incorporation of radiolabel into phospholipid in $P. putida$ Idaho cells grown in the presence and absence of $o$-xylene; (iv) there was a significant difference between the rates of incorporation of radiolabel into phospholipid of $P. putida$ MW1200 and $P. putida$ Idaho grown in the absence of $o$-xylene; and (v) there was a significant difference between the rates of incorporation of radiolabel into phospholipid of $P. putida$ MW1200 and $P. putida$ Idaho grown in the presence of $o$-xylene.

**TABLE 3.** $t$ tests for phospholipid biosynthetic rate data ($\alpha = 0.01$)

<table>
<thead>
<tr>
<th>Rate comparison*</th>
<th>$P$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW1200&lt;sub&gt;s&lt;/sub&gt; vs MW1200&lt;sub&gt;75x&lt;/sub&gt;</td>
<td>0.0512</td>
<td>Not significant</td>
</tr>
<tr>
<td>Idaho&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;75x&lt;/sub&gt;</td>
<td>0.0006</td>
<td>Significant</td>
</tr>
<tr>
<td>Idaho&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>0.0004</td>
<td>Significant</td>
</tr>
<tr>
<td>Idaho&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>0.4596</td>
<td>Not significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;s&lt;/sub&gt;</td>
<td>$&lt;0.0001$</td>
<td>Significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;s&lt;/sub&gt;</td>
<td>$&lt;0.0001$</td>
<td>Significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>$&lt;0.0001$</td>
<td>Significant</td>
</tr>
</tbody>
</table>

* Subscripts: $s$, growth in succinate-minimal salts medium; $75x$, growth in succinate-minimal salts medium plus 75 ppm of $o$-xylene; $200x$, growth in succinate-minimal salts medium plus 200 ppm of $o$-xylene.

Data on the loss of label (turnover) from the phospholipid are shown in Fig. 3. The rate of loss of label from strain MW1200 in the absence of $o$-xylene was 24.89 ($\pm 9.24$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.358$). The rate of loss of label from MW1200 strain in the presence of $o$-xylene was 42.89 ($\pm 11.24$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.5284$). The rate of loss of label from the Idaho strain in the absence of $o$-xylene was 56.44 ($\pm 16.32$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.4793$). The rate of loss of label from the Idaho strain in the presence of 75 ppm of $o$-xylene was 131.7 ($\pm 12.38$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.897$). The rate of loss of label from strain Idaho in the presence of 200 ppm of $o$-xylene was 178.3 ($\pm 18.22$) cpm nmol$^{-1}$ min$^{-1}$ ($r^2 = 0.881$). The rates of loss from both strains in the absence of $o$-xylene were not significantly different from zero. The rates of loss for both strains following exposure to xylene were significantly different from zero.

$t$ tests (Table 4) run on the total phospholipid turnover data showed the following results: (i) for strain MW1200, there was no significant difference between the rates of loss of radiolabel into phospholipid in cells grown in the presence and absence of xylene; (ii) there was no significant difference between the rates of loss of $P. putida$ Idaho and $P. putida$ MW1200 grown in the absence of $o$-xylene; (iii) there was no significant difference between the rates of loss of $P. putida$ Idaho grown in the presence of 75 ppm of $o$-xylene and 200 ppm of $o$-xylene; (iv) there was a significant difference between the rates of incorporation of radiolabel into phospholipid in $P. putida$ Idaho cells grown in the presence and absence of xylene; (v) there was a significant difference between the rates of incorporation of radiolabel into phospholipid of $P. putida$ MW1200 and $P. putida$ Idaho grown in the presence of $o$-xylene.

**TABLE 4.** $t$ tests for rate of loss (phospholipid turnover) data ($\alpha = 0.01$)

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<tr>
<th>Rate comparison*</th>
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<td>Not significant</td>
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<td>Idaho&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;75x&lt;/sub&gt;</td>
<td>0.0014</td>
<td>Significant</td>
</tr>
<tr>
<td>Idaho&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>0.0002</td>
<td>Significant</td>
</tr>
<tr>
<td>Idaho&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>0.8452</td>
<td>Not significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;s&lt;/sub&gt; vs Idaho&lt;sub&gt;s&lt;/sub&gt;</td>
<td>0.0186</td>
<td>Not significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;s&lt;/sub&gt;</td>
<td>0.0014</td>
<td>Significant</td>
</tr>
<tr>
<td>MW1200&lt;sub&gt;75x&lt;/sub&gt; vs Idaho&lt;sub&gt;200x&lt;/sub&gt;</td>
<td>0.0002</td>
<td>Significant</td>
</tr>
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</table>

* Subscripts: $s$, growth in succinate-minimal salts medium; $75x$, growth in succinate-minimal salts medium plus 75 ppm of $o$-xylene; $200x$, growth in succinate-minimal salts medium plus 200 ppm of $o$-xylene.
In the absence of solvent, strain MW1200 showed greatest incorporation of label into phosphatidylglycerol, followed by cardiolipin and phosphatidylethanolamine. Cardiolipin showed the most rapid turnover, followed by phosphatidylglycerol and phosphatidylethanolamine in P. putida MW1200 (Fig. 4A). Phosphatidic acid was detected only at very low levels, and phosphatidylserine was not detected at all. In the presence of o-xylene, cardiolipin showed the greatest incorporation of label, followed by phosphatidylglycerol, phosphatidic acid, and phosphatidylethanolamine (Fig. 4B). Cardiolipin again showed the most rapid turnover, followed by phosphatidic acid, a phosphatidyglycerol precursor. Phosphatidylethanolamine and phosphatidylglycerol showed relatively little turnover following exposure to o-xylene.

In the absence of o-xylene, P. putida Idaho exhibited the greatest incorporation of label into phosphatidylglycerol, followed by cardiolipin and phosphatidylethanolamine. Cardiolipin showed the most rapid turnover, followed by phosphatidylglycerol and phosphatidylethanolamine in P. putida MW1200 (Fig. 5A). Phosphatidic acid was detected only at very low levels, with no phosphatidylserine detected. In the presence of 75 ppm of o-xylene, phosphatidylglycerol showed the greatest incorporation of label, followed by phosphatidic acid, phosphatidylethanolamine, and cardiolipin. Phosphatidic acid showed the greatest turnover, followed by phosphatidylethanolamine (Fig. 5B). Phosphatidylethanolamine and cardiolipin both showed turnover, but at a much lower rate. In the presence of 200 ppm of o-xylene, phosphatidylethanolamine showed the greatest incorporation of label, followed closely by phosphatidylglycerol (Fig. 5C). Cardiolipin and phosphatidic acid also showed incorporation of label. Interestingly, at this concentration, phosphatidylethanolamine showed the greatest turnover, followed by cardiolipin, phosphatidylglycerol, and phosphatidic acid.

**DISCUSSION**

The two P. putida strains were similar in phospholipid composition, harboring typical phospholipids of fluorescent pseudomonads (47). Following exposure to xylene, strain MW1200...
higher in xylene-exposed cells than in cells not exposed to xylene. Membrane phospholipids damaged by xylene must be replaced to maintain membrane integrity. The greater rate of turnover in strain Idaho indicates that this strain may be more efficient at scavenging damaged membrane components for use in phospholipid synthesis.

Analysis of individual phospholipid turnover showed that phosphatidylethanolamine was the most abundant phospholipid in both strains. Therefore, it seems likely that the most incorporation of labeled phosphate would be in phosphatidylethanolamine. However, in strain MW1200, it showed the lowest incorporation and turnover in both the presence and absence of o-xylene. In strain Idaho, phosphatidylethanolamine also showed the least incorporation and turnover in the absence of o-xylene and in the presence of 75 ppm of o-xylene. Only at 200 ppm did phosphatidylethanolamine show significant turnover. It is possible that phosphatidylethanolamine does not incur as much damage as other phospholipids and therefore does not need to be synthesized at as a high a level as the other phospholipids. This may explain why incorporation was high in strain Idaho at 200 ppm and not at 75 ppm.

Interestingly, cardiolipin showed significant turnover in both strains grown in the presence of xylene. This was surprising because cardiolipin constitutes a very low proportion of the total phospholipid in these P. putida strains. Increases in phosphatic acid and phosphatidylglycerol followed decreases in cardiolipin specific activity, indicating that phosphatidylglycerol is synthesized at the expense of cardiolipin. The fact that the amount of cardiolipin proportionally decreases in xylene-exposed cells (in both strains) supports this conclusion.

In both strains, phosphatidylglycerol generally showed the greatest incorporation of label. It also showed a higher rate of turnover than phosphatidylethanolamine in all cases except P. putida Idaho in the presence of 200 ppm of o-xylene. Although this is the second most abundant phospholipid in each strain, it accounts for only 25% or less of the total phospholipid in both strains. Because it shows high incorporation of label and significant turnover, it is likely that this phospholipid incurs more damage by xylene than phosphatidylethanolamine and needs to be replaced more rapidly. This could also account for the rapid turnover and proportional decrease in cardiolipin. The turnover of cardiolipin, which contains two molecules of phosphatidylglycerol, is an efficient method to replenish phosphatidylglycerol pools.

It is apparent that the solvent-tolerant strain studied here has a statistically significant increased biosynthetic rate following exposure to o-xylene. This indicates that an inducible membrane repair mechanism which allows rapid repair of damaged membrane components exists in P. putida Idaho. Investigators of solvent-tolerant organisms have noted an increased requirement for magnesium for these strains. Ramos et al. (35) and Inoue et al. (20) showed that the addition of excess magnesium ions aided in decreasing the lag phase for cells grown in high concentrations of toluene. If rapid membrane repair occurs in the solvent-tolerant strains, then a requirement for magnesium ions would exist because magnesium is known to be one of the important cations in phospholipid and LPS stabilization (25).

While an increased rate of membrane biosynthesis in response to cell damage has not been previously reported, rapid repair of damaged cell components as a resistance mechanism is not an unusual phenomenon. Deinococcus radiodurans, for example, has a DNA polymerase that is 100 to 150 times as efficient as that found in E. coli. This attribute allows the species to survive high doses of ionizing radiation (42). Resistance to the antibiotic trimethoprim has been acquired by some bacterial strains by overproducing the target molecule of the antibiotic, dihydrofolate reductase, by 600 times the nor-
mal level, thus negating the effect of trimethoprim. In Staphylococcus aureus, resistance to methicillin is acquired through overproduction of PBP2a (11). In E. coli, a mutation in cardiolipin synthesis conferred resistance to 3,4-dihydroxybutyl 1-phosphonate (17).

Although many investigators have examined fatty acid content and phospholipid content of various bacterial strains in response to organic solvents, to date no one has examined phospholipid biosynthetic rates following exposure to organic solvents. It has been shown that P. putida Idaho demonstrates an increased rate of phospholipid biosynthesis following exposure to the organic solvent o-xylene, a response not shown in a similar but solvent-sensitive strain, P. putida MW1200. This energy-dependent process promotes repair of membranes damaged by solvents and appears to enhance tolerance to high concentrations of organic solvents in P. putida Idaho.

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REFERENCES