Construction and Characterization of a 1,3-Propanediol Operon

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1,3-Propanediol (1,3-PD) is a three-carbon diol that is currently manufactured by synthetic processes beginning with petroleum derivatives such as acrolein or ethylene oxide (35). An emerging large-volume application of 1,3-PD is as a monomer for the production of polyesters. The 1,3-PD operon was designed so that it can be readily modified for expression in other prokaryotic hosts; therefore, it is useful for metabolic engineering of 1,3-PD pathways from glycerol and other substrates such as glucose.

The genes for the production of 1,3-propanediol (1,3-PD) in Klebsiella pneumoniae, dhaB, which encodes glycerol dehydratase, and dhaT, which encodes 1,3-PD oxidoreductase, are naturally under the control of two different promoters and are transcribed in different directions. These genes were reconfigured into an operon containing dhaB followed by dhaT under the control of a single promoter. The operon contains unique restriction sites to facilitate replacement of the promoter and other modifications. In a fed-batch cofermentation of glycerol and glucose, Escherichia coli containing the operon consumed 9.3 g of glycerol per liter and produced 6.3 g of 1,3-PD per liter. The fermentation had two distinct phases. In the first phase, significant cell growth occurred and the products were mainly 1,3-PD and acetate. In the second phase, very little growth occurred and the main products were 1,3-PD and pyruvate. The first enzyme in the 1,3-PD pathway, glycerol dehydratase, requires coenzyme B₁₂, which must be provided in E. coli fermentations. However, the amount of coenzyme B₁₂ needed was quite small, with 10 nM sufficient for good 1,3-PD production in batch cofermentations. 1,3-PD is a useful intermediate in the production of polyesters. The 1,3-PD operon was designed so that it can be readily modified for expression in other prokaryotic hosts; therefore, it is useful for metabolic engineering of 1,3-PD pathways from glycerol and other substrates such as glucose.

1,3-Propanediol (1,3-PD) is a three-carbon diol that is currently manufactured by synthetic processes beginning with petroleum derivatives such as acrolein or ethylene oxide (35). An emerging large-volume application of 1,3-PD is as a monomer for the production of polyesters. The 1,3-PD operon was designed so that it can be readily modified for expression in other prokaryotic hosts; therefore, it is useful for metabolic engineering of 1,3-PD pathways from glycerol and other substrates such as glucose.

This novel genetic configuration provides the basis for an improved microbial 1,3-PD process. Several research groups have achieved 1,3-PD concentrations of 60 to 70 g/liter in the fermentation of glycerol, using organisms that can naturally convert glycerol to 1,3-PD (13, 18, 29, 30). Without directed improvement of the host, however, this level of performance is probably a plateau and cannot compete with newly improved synthetic processes. In 1995, Shell Chemical Company announced an improvement to the ethylene oxide hydrocarbonylation process that permits 1,3-PD to be produced at a cost low enough for its use in polypropylene terephthalate carpet fibers (32). Metabolic engineering provides a means to improve the fermentation process. DuPont, for example, recently patented a process to convert sugars to 1,3-PD with various organisms expressing glycerol dehydratase and 1,3-PD oxidoreductase from K. pneumoniae (24). The operon we describe in this report is designed so that any promoter and other desired genetic elements can be readily introduced to enable expression of the 1,3-PD genes in various prokaryotes. As such, it should prove useful in the metabolic engineering of 1,3-PD processes.

MATERIALS AND METHODS

DNA sequencing. Sequencing of the K. pneumoniae DNA was performed by the dideoxy chain termination method (31) with a Sequenase 2.0 kit (United States Biochemicals, Cleveland, Ohio) at Lofstrand Labs Limited (Gaithersburg, Md.). Two large pieces of DNA to be sequenced, Apal-SacI and Nhel-Apal fragments, were separately cloned into the vector pSL301 (Invitrogen, San Diego, Calif.). This vector contains a multiple cloning site flanked by T7 and T3 promoter sequences; therefore, T3 and T7 primers were used to initiate double-stranded sequencing. Primer walking with synthetic 18-mer oligonucleotide primers was used to determine the remainder of the double-stranded sequence, with a new primer synthesized for about every 250 nucleotides sequenced.

The high GC content of the sequenced DNA resulted in numerous compressions, which were resolved by the inclusion of 25 or 40% formamide in the sequencing gel or the substitution of 7-deaza-dGTP for dGTP. Artifact banding
Bacterial strains, media, and growth conditions. *K. pneumoniae* was obtained from the American Type Culture Collection (Rockville, Md.) as strain ATCC 25955. The *E. coli* strains used in all fermentations were AG1 (Stratagene, La Jolla, Calif.) and TOP10F* (Invitrogen). The temperature for all fermentations and induction cultures was 37°C. The volumes of the fed-batch fermenter were 4 liters. The volumes of all other fermentations were 300 ml when extracts were prepared or 2 ml when they were not. The fed-batch fermentation was conducted in a BioFlo 3000 fermentor (New Brunswick Scientific, Edison, N.J.) with pH controlled at 7.0, agitation at 50 rpm, and nitrogen sparging to minimize dissolved oxygen. Fermentations of the 300-ml total volume were conducted without agitation for at least 10 h in anaerobic flasks. Two-milliliter fermentations were conducted in closed screw-cap tubes with a total liquid volume of 1.8 ml for at least 10 h. Inocula for smaller-scale fermentations were started from stocks frozen in glycerol and grown overnight with shaking in 2 ml of Luria-Bertani medium plus 100 µg of ampicillin per ml (LA medium). The two-milliliter fermentation mixtures were inoculated with 50 µl of the overnight culture, and 300-ml fermentation mixtures were inoculated with 500 µl. The inoculated 4-liter fermentation was grown as for the smaller-scale fermentations, and then 1,3-PD oxidoreductase was assayed by the method of Johnson and Lin (21). The initial rate of reduction of NADH was measured spectro-photometrically at 25°C for a final concentration containing 100 mM 1,3-PD, 35 mM ammonium sulfate, 100 mM potassium bicarbonate buffer (pH 9.0), 0.6 mM NADH, and 10 to 50 µl of crude cell extract in a final volume of 1 ml. A baseline was established prior to the addition of NADH. Glycerol dehydrogenase activity was measured by a coupled assay with yeast alcohol dehydrogenase (42) or by the MBTH (3-methyl-2-benzothiazoline hydrazone) method (43).

Preparation of cell extracts. Crude cell extracts were prepared by sonication of cell pastes and subsequent centrifugation. Cell pastes were obtained by centrifuga-
tion of fermentation broths at 4,000 rpm for 5 to 10 min at 4°C with a Beckman (Fullerton, Calif.) model J-21 centrifuge and JA-20 rotor. The pastes were washed in 20 mM Tris buffer (pH 8.0) or 50 mM potassium phosphate buffer (pH 8.0), centrifuged as described above, and resuspended in a small amount of the appropriate assay resuspension buffer. The cells were then disrupted by sonication for 5 min on ice at a duty cycle of 70% with 1-s cycles. Cell debris removed by centrifugation at maximum speed for 5 to 15 min in a microcentrifuge.

Assays. 1,3-PD oxidoreductase was assayed by the method of Johnson and Lin (21). The initial rate of reduction of NADH to NAD was measured spectrophotometrically at 25°C for a final concentration containing 100 mM 1,3-PD, 35 mM ammonium sulfate, 100 mM potassium bicarbonate buffer (pH 9.0), 0.6 mM NADH, and 10 to 50 µl of crude cell extract in a final volume of 1 ml. A baseline was established prior to the addition of NADH. Glycerol dehydrogenase activity was measured by a coupled assay with yeast alcohol dehydrogenase (42) or by the MBTH (3-methyl-2-benzothiazoline hydrazone) method (43).

For the above assays, 1 U is defined as the number of micromoles of NADH reduced, NADH oxidized, or propionaldehyde formed per minute at the assay temperature. Total protein concentrations in cell extracts were determined by using the Bradford assay kit (Bio-Rad, Hercules, Calif.) with bovine serum albumin as the standard.

High-performance liquid chromatography analysis. The metabolites present in fermentation broths were analyzed with a Bio-Rad high-performance liquid chromatography system with a refractive index detector and a Bio-Rad Aminex HPX-87H organic acids column at a flow rate of 0.6 ml/min and a column temperature of 65°C. The mobile phase was 0.01 N sulfuric acid. Samples were filtered through 0.45-µm-pore-size Supor membranes (Gelman Sciences, Ann Arbor, Mich.) prior to analysis.

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done with a Hoefer (San Francisco, Calif.) SE 600 vertical unit. Each protein sample was diluted with an equal volume of loading buffer (20% [vol/vol] glycerol, 2% [vol/vol] β-mercaptoethanol, 120 mM Tris-Cl [pH 6.8], 41 mg of SDS per ml, 0.01 mg of bromophenol blue per ml) and boiled for 5 min prior to being loaded onto a 1.5-mm-thick gel. The stacking gel and 10% separating gel were prepared as described by Ausubel et al. (4). The gel was run at 20 mA until the blue dye entered the separating gel, and subsequently it was run at 30 mA for 3 h.

### TABLE 1. Cloning vectors used and plasmids cited in the text

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Properties</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322</td>
<td><em>E. coli</em> cloning vector</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pSE280</td>
<td>trc promoter upstream of MCS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pSE380</td>
<td>pSE280 with constitutive lac repressor gene</td>
<td>In Vitrogen</td>
</tr>
<tr>
<td>pSL301</td>
<td>lac promoter upstream of MCS</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pTC1</td>
<td>Cosmid containing <em>K. pneumoniae</em> dhaB, dhaT, dhaD, and dhaK genes</td>
<td>This work</td>
</tr>
<tr>
<td>pTC3</td>
<td>12.1-kb HaeIII-SacI fragment of pTC1 inserted into pBR322</td>
<td>This work</td>
</tr>
<tr>
<td>pTC9</td>
<td>230-bp EcoRI-Nhel fragment deleted from pTC3 and remaining HindIII site</td>
<td>This work</td>
</tr>
<tr>
<td>pTC42</td>
<td>2.3-kb fragment of pTC9 from start of ORF 2 (dhaT) to SacI site inserted</td>
<td>This work</td>
</tr>
<tr>
<td>pTC48</td>
<td>1,3-PD operon; unidirectional transcription of dhaB and dhaT from trc promoter; construction described in Materials and Methods</td>
<td>This work</td>
</tr>
<tr>
<td>pTC49</td>
<td>pTC48 with 596-bp SalI-NoI fragment replaced by 677-bp PCR-derived ORF 4</td>
<td>This work</td>
</tr>
<tr>
<td>pTC50</td>
<td>pTC48 with 596-bp SalI-NoI fragment replaced by 603-bp PCR-derived ORF 4</td>
<td>This work</td>
</tr>
<tr>
<td>pTC53</td>
<td>pTC50 with lacP&lt;sup&gt;b&lt;/sup&gt; gene inserted at SacI site</td>
<td>This work</td>
</tr>
<tr>
<td>pTC63</td>
<td>1.5-kb NruI-NoI fragment (within ORF 3) deleted from pTC49</td>
<td>This work</td>
</tr>
<tr>
<td>pTrclB1</td>
<td>5.3-kb KpnI-Nhel fragment of pTC9 inserted into pSE280</td>
<td>This work</td>
</tr>
</tbody>
</table>

<sup>a</sup> MCS, multiple cloning site.
hybridize to regions of pTC9 further upstream of ORF 4 than the primer used in the construction of pTC48. pTC53 is identical to pTC50 except that the constitutive lac repressor, isolated by PCR from the vector pSE380, was inserted into the SacI site so that the lac repressor is transcribed in the same direction as the 1,3-PD genes.

pTrcB1 was constructed by ligating the KpnI-NheI fragment of pTC9 into pSE280 (Invitrogen) cut with KpnI and SpeI.

Nucleotide sequence accession number. The nucleotide sequence described in this paper was submitted to GenBank under accession no. U30903.

RESULTS

General features of the DNA sequence. The sequence of an NheI-SacI fragment of cosmid pTC1 (41), a contiguous sequence of 8,067 nucleotides, was determined as described in Materials and Methods. Several large ORFs (that would encode proteins of 10 kDa or more) were found within the fragment known to contain the dhaB and dhaT genes. The major ORFs were designated as shown in Fig. 2.

Identification of functional units conferring dhaB and dhaT activities. The main objective in the construction of the 1,3-PD operon was to enable expression of the dhaB and dhaT genes in one transcript under the regulation of a single replaceable promoter/operator. The construction of the 1,3-PD operon therefore required the identification of regions of DNA that confer dhaB and dhaT activities but are independent of their native regulation.

Glycerol dehydratase (DhaB) is known to consist of multiple subunits. This suggests that the DNA encoding it consists of multiple ORFs. Stroinski et al. (34) reported that the active DhaB enzyme consists of two subunits, A (22 kDa), which itself dissociates into two subunits with an apparent molecular mass of about 12 kDa each, and B (189 ± 22 kDa). It was shown that subunit B could be further dissociated into subunits of 90 ± 25 kDa in the presence of 0.1 M KCl. A portion of the sequenced fragment, the KpnI-NheI fragment, was found to be sufficient for dhaB activity. This fragment contains ORFs transcribed in
the following order: 4, 4a, 3a, 3. Plasmid pTrcB1 contains the Kpnl-Nhel fragment under control of the trc promoter. Crude cell extracts of E. coli/pTrcB1 possessed glycerol dehydrogenase activity. Living E. coli/pTrcB1 grown in the standard medium, except with glucose and glycerol replaced by xylose and 1,2-propanediol (1,2-PD), converted 1,2-PD to 1-propanol only when coenzyme B$_{12}$ was added to the medium. K. pneumoniae glycerol dehydrogenase can accept 1,2-PD as a substrate, and the conversion of 1,2-PD to 1-propanol is analogous to the conversion of glycerol to 1,3-PD. 1,2-PD was used in place of glycerol to avoid accumulation of highly toxic 3-HPA, and xylose was used instead of glucose to avoid possible catabolite repression of the endogenous dehydrogenase responsible for conversion of propionaldehyde to 1-propanol.

1,3-PD oxidoreductase (DhaT) is reported (12, 21) to consist of a single subunit of 40 to 45 kDa whose active form comprises an octamer of this subunit. We cloned ORF 2 downstream of the trc promoter and included a copy of the constitutive lac repressor gene to form plasmid pTC42. We compared dhaT activity levels in extracts of induced and uninduced E. coli/pTC42 cells. We also compared these to the activities in extracts of E. coli cells containing pTC9, a plasmid that allows significant 1,3-PD synthesis, to ensure that pTC42 was capable of conferring adequate dhaT activity. When pTC42 fermentation cultures were uninduced, the resulting cell extracts possessed no detectable dhaT activity. pTC42 fermentation mixtures that contained 0.5 mM IPTG throughout gave cell extracts with a dhaT activity (0.8 U/mg of protein) twice that of pTC9 cell extracts (0.4 U/mg of protein). ORF 2 was expected to encode a protein with a molecular weight of 41,459 based on translation of the nucleotide sequence. SDS-PAGE of induced and uninduced pTC42 extracts showed that induced cells overproduced a protein of just under 43 kDa, whereas uninduced cells did not (Fig. 3).

Comparison of identity at the amino acid level provided further evidence that ORF 2 encodes 1,3-PD oxidoreductase. ORF 2 is homologous to a number of NAAD-dependent oxidoreductases having identities at the amino acid level of from 36% with E. coli alcohol:NAD$^+$ oxidoreductase (AdhE) to 94% with C. freundii 1,3-PD:NAD$^+$ oxidoreductase (DhaT). Daniel et al. (11) have reported the homology of the C. freundii DhaT protein to a number of type III oxidoreductases. The gene product of ORF 2, like the type III oxidoreductases, contains the characteristic iron-containing-protein signature GxxHxxAXxxGxxxxxPHG (5).

The above evidence led to the assignment of the regions of DNA to be considered functional dhaB and dhaT units for the purpose of operon construction. For dhaB, the unit is the set of ORFs to be transcribed in the order 4, 4a, 3a, 3. For dhaT, the unit is ORF 2.

Construction of the 1,3-PD operon. In the native DNA fragment isolated from K. pneumoniae the dhaT and dhaB genes are transcribed in opposite directions from a common region. In Fig. 2, ORF 2 (dhaT) is transcribed from left to right, and ORFs 4, 4a, 3a, and 3 are transcribed from right to left. Therefore, the steps necessary for construction of the 1,3-PD operon were (i) isolation of the functional dhaB and dhaT units without their promoters, (ii) rearrangement of the DNA so that the two units are transcribed in the same direction, and (iii) attachment of a replaceable promoter upstream of the units. A representation of the 1,3-PD operon (plasmid pTC49) is shown in Fig. 4.

Construction of the 1,3-PD operon was accomplished by PCR, restriction digestions, and ligations as described in Materials and Methods. ORFs 2 and 4 were amplified by PCR from the beginning of the ORF to a site downstream of an internal restriction site and religated to the remainder of the corresponding gene. The trc promoter (with the lac repressor binding site but no catabolite gene activator protein-cyclic AMP binding site) was isolated by PCR from pSE380 so that the amplification product would be bounded by BgII sites. The promoterless genes and the trc promoter were reassembled into pBR322 so that the trc promoter directs monocistronic transcription of the ORFs in the order 4, 4a, 3a, 3, 2.

The significance of ORF 3. Tobimatsu et al. (38) reported that ORF 3 does not encode a subunit of glycerol dehydrogenase. We observed a loss of dehydrogenase activity when the SfiI site within ORF 3 of pTC9 was disrupted by digestion and blunt-end ligation (44). The relevance of ORF 3 to the 1,3-PD operon was tested by conducting fermentations with strain TOP10$^{F}$ carrying either pTC49 or pTC63 (pTC49 with an NruI-NruI deletion within ORF 3). TOP10$^{F}$/pTC63 produced about 40% as much 1,3-PD as TOP10$^{F}$/pTC49 (Table 2), indicating that ORF 3 is not necessary for glycerol dehydrogenase activity, but it may serve some other function that permits 1,3-PD synthesis to be more effective.

Requirement for coenzyme B$_{12}$ addition. We determined to what extent coenzyme B$_{12}$ addition was necessary to effect 1,3-PD production in the transgenic E. coli cells. Figure 5 shows the final 1,3-PD concentrations of fermentations in which TOP10$^{F}$/pTC49 was grown in the standard 1,3-PD production medium except with various low concentrations of coenzyme B$_{12}$. 1,3-PD was not formed by TOP10$^{F}$/pTC49 when coenzyme B$_{12}$ was not added, and TOP10$^{F}$ cells did not synthesize 1,3-PD without the K. pneumoniae genes, confirming that the
cloned *K. pneumoniae* genes are responsible for 1,3-PD synthesis. Coenzyme B$_12$ was no longer limiting to 1,3-PD synthesis when provided at concentrations above 10 nM, far below the concentration in our standard production medium (1 μM).

**Fed-batch fermentation.** We conducted a 4-liter fed-batch cofermentation of glycerol and glucose with *E. coli* AG1/pTC53 without induction to determine what concentration of 1,3-PD could be achieved in the final broth. pTC53 is essentially the same as pTC49 except that it contains the constitutive lac repressor gene (*lacI*). pTC49, although it can be somewhat more effective in 1,3-PD synthesis, was not used because of its tendency to rearrange. pTC49 and pTC53 are stable plasmids, but pTC49 often did not retain its original configuration over many generations. It is possible that the addition of the *lacI* gene to pTC49 prevents excessive transcription and that rearrangement in pTC53 does not provide a significant growth advantage. Figure 6 shows the time course of the AG1/pTC53 fermentation, in which glucose and glycerol were adjusted to 2 g/liter each time the concentrations of both components were nearing zero. The results of the fermentation are summarized in Table 3. The final concentration of 1,3-PD was 6.3 g/liter. The fermentation had two distinct phases, one in which the cells grew and produced predominantly 1,3-PD and acetate, and another after the cells ceased to grow in which pyruvate became the dominant acid product.

**DISCUSSION**

We have constructed an operon containing the glycerol dehydratase and 1,3-PD oxidoreductase genes of *K. pneumoniae*.

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**FIG. 4.** Plasmid pTC49, one version of the 1,3-PD operon. The regions sufficient for glycerol dehydratase (*dhaB*) and 1,3-PD oxidoreductase (*dhaT*) expression (black segments) and the direction of transcription (arrows) are indicated. The *ampR* (ampicillin resistance) gene and the pMB1 origin of replication are identical to those in pBR322. Nucleotide positions are also indicated (in parentheses).

**TABLE 2.** Production of 1,3-PD in batch culture by TOP10F'/pTC49 and TOP10F'/pTC63

<table>
<thead>
<tr>
<th>Initial glycerol concn (g/liter)</th>
<th>1,3-PD produced (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOP10F'/pTC49</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>5</td>
<td>1.19</td>
</tr>
<tr>
<td>10</td>
<td>1.18</td>
</tr>
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</table>

* Fermentations were carried out in the standard 1,3-PD production medium in closed 2-ml screw-cap tubes with no agitation for 15 h at 37°C. pTC63 is identical to pTC49 except that it lacks the NruI-NruI fragment (1.5 kb) within ORF 3.

**FIG. 5.** 1,3-PD production in *E. coli* TOP10F' carrying pTC49. The 2-ml fermentations were carried out as described in Materials and Methods in standard 1,3-PD production medium but with various low concentrations of coenzyme B$_12$. Symbols: □, final 1,3-PD concentration (in grams per liter); ■, molar yield of 1,3-PD from glycerol (moles of 1,3-PD produced per mole of glycerol consumed).
The operon enables the production of 1,3-PD in *E. coli* and provides the basis for future expression of the 1,3-PD pathway under novel regulation in other organisms. It also provides a basis for extending the 1,3-PD pathway to include fermentable sugars. Laffend et al. (24) have already demonstrated that the sugars-to-1,3-PD pathway is realizable. Expression of the 1,3-PD genes in an organism that can naturally produce glycerol from sugars would complete a microbial sugars-to-1,3-PD pathway, averting the possibly toxic effects of high glycerol concentrations and providing a microbial route to 1,3-PD from fermentable sugars, which are more abundant, less expensive, and utilizable by a wider range of organisms than glycerol.

The 1,3-PD operon is very flexible in that it contains unique restriction sites in several strategic locations (Fig. 4). The promoter region can be replaced with any *Bgl*II-*Bgl*II fragment; PCR can be used to isolate any promoter region of interest bounded by *Bgl*II sites, and the promoter region can be ligated into this location. We observed that the *trc* promoter fragment could be replaced by the *E. coli lac* or *phoA* promoter with the retention of the ability to produce 1,3-PD, but removal of the promoter caused a dramatic decrease in 1,3-PD production (data not shown). The *Bgl*II sites flanking the promoter are also significant because DNA cleaved with *Bgl*II can be ligated to DNA cleaved with *Sau*3AI without the creation of blunt ends with DNA polymerase. Therefore, *Sau*3AI digests of genomic DNA of any organism can be randomly ligated into this location to screen for promoters effective in the production of 1,3-PD under any conditions desired, an implementation not possible with unmanipulated *K. pneumoniae* DNA. The upstream regions of ORF 2 or 4 can be replaced with any *KpnI-MluI* or *SalI-NorI* fragment, respectively. This allows the introduction of different ribosome binding sites or leader sequences at either of these locations. Additional genes, promoters, and terminator structures can be ligated into the operon before or after the promoter, dehydratase region, or oxidoreductase region.

During the construction of the 1,3-PD operon, we determined that ORFs 4, 4a, 3a, and 3 are sufficient for glycerol dehydratase activity. Two important points concerning this determination should be addressed here: the demonstration of the activity and the significance of ORF 3. We observed that cells containing pTrcB1 (ORFs 4, 4a, 3a, and 3 under control of the *trc* promoter) possessed glycerol dehydratase activity. These cells converted 1,2-PD to 1-propanol when the medium was supplemented with coenzyme B12. Glycerol dehydratase converts glycerol to 3-HPA, which is a potent antimicrobial agent (15, 36), but it converts 1,2-PD to propionaldehyde, which is less toxic to *E. coli* than is 3-HPA (15, 23). We therefore used 1,2-PD as a substrate instead of glycerol to demonstrate the activity in whole cells.

Tobimatsu et al. (38) reported that the *K. pneumoniae* ORF corresponding to our ORF 3 is not necessary for glycerol dehydratase activity. We found this to be true also, but we found that ORF 3 seems to augment 1,3-PD synthesis by providing an unknown function. ORF 3 has some identity to coenzyme B12-dependent enzymes. A generally recognized conserved sequence in such enzymes is DxHxxG (7). This motif is not encoded in ORF 3; however, the translation of ORF 3 shares a VGxSSL motif with the coenzyme B12-dependent enzymes methylmalonyl-coenzyme A mutase (8, 28), methy-
an octamer of a single subunit (ORF 2), it may be advantagous because neither of the two known energy because neither of the two known sites of various strengths or even a second promoter up-stream of dhaT. The flexible construction of the 1,3-PD operon allows such changes to be made readily.

Theoretically, 1 mol of 1,3-PD can be produced for every mol of glycerol consumed, given that the cells are provided with a cosubstrate such as glucose from which to derive reducing power (40). We observed a yield of 82% in the AG1/pTC53 fermentation, which means that 18% of the glycerol consumed was not converted to 1,3-PD. In the absence of respiration, E. coli cannot use glycerol as the sole source of carbon and energy because neither of the two known 1,3-PD could lead to higher concentrations of 1,3-PD than are currently possible.

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REFERENCES


Shell Chemical Company. 9 May 1995. Shell Chemical Company announces commercialization of new polymer. (Press release.)


