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Accumulation of Methylglyoxal in Anaerobically Grown *Escherichia coli* and Its Detoxification by Expression of the *Pseudomonas putida* Glyoxalase I Gene

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Anaerobic glycerol fermentation by *Escherichia coli* strains expressing genes from the *Klebsiella pneumoniae dha* regulon showed that cell growth and 1,3-propanediol (1,3-PD) production are significantly inhibited when 5 g/L or higher of glycerol is initially present. One reason for this inhibition may be methylglyoxal (MG) accumulation. Assays of both intracellular and extracellular MG levels indicated an accumulation of MG in anaerobic glycerol fermentation of transgenic *E. coli*. *Pseudomonas putida* glyoxalase I was expressed in the transgenic *E. coli* to enhance MG detoxification. The activity of glyoxalase I in the transgenic *E. coli* with the *P. putida* glyoxalase I under anaerobic conditions was 12-fold higher than that in the control cells. Compared to the control cells, the transgenic cells with the *P. putida* glyoxalase I displayed a reduction of 35–43% in intracellular MG and a decrease of 30% in extracellular MG. These decreases were statistically significant ($P > 94$). Furthermore, the expression of the *P. putida* glyoxalase I in the transgenic *E. coli* markedly improved cell growth and resulted in a 50% increase in 1,3-PD production.

INTRODUCTION

1,3-Propanediol (1,3-PD) is an emerging industrial chemical that has application in the production of polymethylene terephthalate and the manufacture of polyurethanes and cyclic compounds. It is currently produced from petroleum derivatives such as ethylene oxide and acrolein by chemical routes. The microbial production of 1,3-PD has recently received much attention because of two advantages. First, it can use renewable feedstocks such as glycerol and sugars, as opposed to nonrenewable petroleum derivatives. Second, with advances in metabolic engineering, the microbial routes have the potential to be economically competitive with the synthetic routes.

Strains of *E. coli* that are capable of converting glycerol to 1,3-PD were previously constructed in our laboratory by importing the *dha* regulon of *K. pneumoniae* into *E. coli* (Tong, 1992; Tong *et al*., 1991). Genes in the *dha* regulon of *K. pneumoniae* have also been characterized and rearranged into a synthetic operon (Skraly, 1997; Skraly *et al*., 1998). The synthetic operon is free of *K. pneumoniae* regulatory elements and allows the 1,3-PD genes to be expressed from a single promoter. However, the conversion of glycerol, which can be utilized by the resulting *E. coli* transformed with either the *K. pneumoniae dha* regulon or the synthetic 1,3-PD operon, is low. Anaerobic glycerol fermentation by the transgenic *E. coli* showed that cell growth and 1,3-PD production were inhibited when the initial concentration of glycerol was 5 g/L or higher (Skraly, 1997; Tong, 1992).

A number of toxic by-products are known to inhibit microbial production of 1,3-PD from glycerol. The most studied of these are acetic acid, butyric acid, and 3-hydroxypropionaldehyde (Barbirato *et al*., 1996; Biebl, 1991). We have previously demonstrated that 1,3-PD production by transgenic *E. coli* can be improved by reducing the accumulation of glycerol 3-phosphate (Zhu and Cameron, 1998; Zhu, 1999). Here we report on the role of a lesser-known metabolite in the pathways related to 1,3-PD production—methylglyoxal (MG), a metabolite produced enzymatically or nonenzymatically from the glycolytic intermediate dihydroxyacetone phosphate (DHAP).

MG is an α-ketoaldehyde that is produced at low levels in nearly all cells (Chaplen *et al*., 1996). It is a toxic compound that, at millimolar concentrations, arrests cell growth (Murata *et al*., 1989). The synthesis of MG serves to relieve the inhibition that would occur if sugar phosphates accumulated (Anderson and Cooper, 1970; Hopper and Cooper, 1972; Tötemeyer *et al*., 1998). MG synthase is an allosterically modulated enzyme subject to feedback inhibition by phosphate and homotropic activation by DHAP (Hopper

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and Cooper, 1971). Thus, the synthesis of MG favors low phosphate and high DHAP. A common reason for MG accumulation is imbalance of metabolism, when formation of DHAP is faster than degradation of MG (Hopper and Cooper, 1971; Maclean *et al.*, 1998). At least two reports showed that MG accumulation occurred in *E. coli* when cells were grown aerobically on glycerol (Cooper and Anderson, 1970; Freedberg *et al.*, 1971). MG halts cell proliferation in *E. coli* at concentrations of 0.2–1.0 mM and leads to growth arrest in leukemia 60 cells *in vitro* (Együd and Szent-Györgyi, 1966; Kang *et al.*, 1996).

The major source of MG in bacteria is enzymatic conversion of DHAP catalyzed by MG synthase (EC 4.2.99.11) (Cooper, 1984). There are at least five different known route for MG degradation involving the following reactions: (i) Glutathione (GSH)-dependent glyoxalases I and II; (ii) GSH-dependent glyoxalase III; (iii) NADPH-dependent MG reductase and NADH-dependent lactaldehyde dehydrogenase; (iv) NADPH-dependent aldose reductase; and (v) MG dehydrogenase, as shown in Fig. 1 (Inoue and Kimura, 1995). Even though there are several ways to degrade MG in a microorganism, the dominant route for detoxification of MG in *E. coli* is the glutathione-dependent glyoxalase I pathway (Maclean *et al.*, 1998). In this pathway, glyoxalase I (EC 4.4.1.5) converts MG and reduces glutathione into *S*-D-lactoylglutathione. Then, glyoxalase II (EC 3.1.2.6) hydrolyzes *S*-D-lactoylglutathione to D-lactic acid and regenerates reduced glutathione.

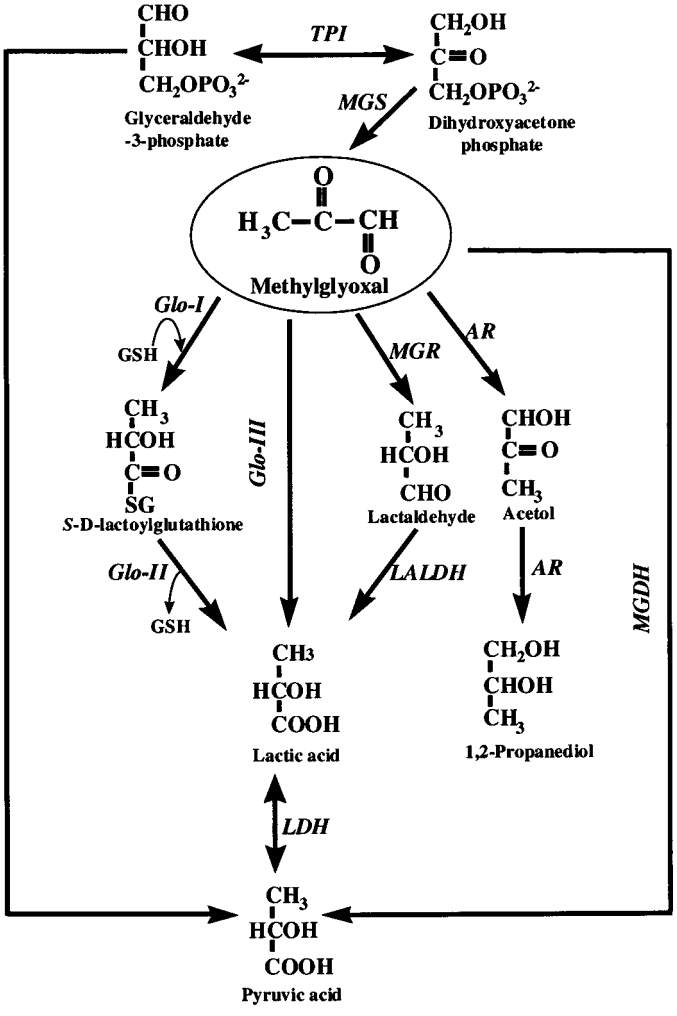

FIG. 1. Major pathways of MG formation and detoxification in a microorganism. Abbreviations used: AR, aldose reductase; Glo-I, glyoxalase I; Glo-II, glyoxalase II; Glo-III, glyoxalase III; GSH, glutathione; LALDH, lactaldehyde dehydrogenase; LDH, lactate dehydrogenase; MGDH, methylglyoxal dehydrogenase; MGR, methylglyoxal reductase; MGS, methylglyoxal synthase; TPI, triosephosphate isomerase.

In this study, the intracellular and extracellular concentrations of MG during anaerobic glycerol fermentation by transgenic *E. coli* are examined. Next, the expression of *Pseudomonas putida* glyoxalase I in *E. coli* is described and the MG detoxification abilities in the resulting cells are characterized by comparing the intracellular and extracellular levels of MG in these transformed cells to those in control *E. coli* with only wild-type glyoxalase I. Finally, cell growth, 1,3-PD production, and MG resistance of *P. putida* glyoxalase I-producing cells are compared to the control cells. The questions of whether MG has a significant effect on cell growth and 1,3-PD production under anaerobic conditions and of whether the glyoxalase I of *P. putida* (an aerobic organism) can be useful under anaerobic conditions are addressed.

MATERIALS AND METHODS

Bacteria, plasmids, and enzymes. *E. coli* AG1(F^- *endA1 hsdr17* [k_n^-, m_k^+] *supE44 thi-1 recA1 gyrA96 relA1* λ^-) (Stratagene, La Jolla, CA) was the strain initially used as the host for 1,3-PD production studies. Three plasmids were used in this work. pTC9, a plasmid of 15.9 kb containing the *dha* regulon from *K. pneumoniae* and an IPTG-inducible promoter, was used for 1,3-PD production in *E. coli* (Tong, 1992). pGI423, a plasmid of 6.2 kb containing the gene responsible for the glyoxalase I activity from *P. putida*, was used as the source for DNA of glyoxalase I activity (Rhee *et al.*, 1987). pTC13, a plasmid including both the *dha* regulon from *K. pneumoniae* and the glyoxalase I gene from *P. putida*, was constructed by inserting the *P. putida* glyoxalase I gene into pTC9 at its *Sal*I site.

Media and growth conditions. Anaerobic fermentations were carried out in 300-ml anaerobic flasks as previously described (Lamed and Zeikus, 1980; Tong *et al.*, 1991). The

medium contained 6 g Na_2HPO_4, 3 g KH_2PO_4, 2 g NH_4Cl, 0.5 g NaCl, 2 mmol $MgSO_4$, 0.5 mg coenzyme B_{12}, 5 g yeast extract, and 100 mg ampicillin in 1 liter of deionized water. Unless otherwise specified, 5 g/L of glycerol was used as carbon source for 1,3-PD production, and 5 g/L of glucose was added to provide more reducing power and to be used as a cosubstrate. The temperature of all fermentations was 37° C. Inocula for all fermentations were started from stocks frozen in glycerol and grown overnight with shaking in LB medium plus 100 mg/L of ampicillin. A total of 1% of inoculum was used for each fermentation. LB medium was made following the procedure of Maniatis *et al.* (1982).

Preparation of cell-free extracts. Cells from late exponential phase were harvested by centrifugation at 4500 rpm for 15 min at 4° C with a Beckman (Fullerton, CA) Model J2-21 centrifuge and JA-20 rotor. The cell pellets were washed with a cold solution of 1% NaCl and stored at −20° C. For disruption, the thawed pellets were resuspended in 1 to 2 ml of 0.1 M potassium phosphate buffer (pH 7.5) and treated by sonication for 5 min on ice at a duty cycle of 60% with 1-s cycles. Cell debris was removed by centrifugation as described above. The resulting supernatant was assayed for the presence of glyoxalase I or intracellular MG. Protein was determined by using the RC DC Protein Assay Kit II from Bio-Rad (Hercules, CA). The assay is based on the color change of Coomassie brilliant blue G-250 dye in response to various concentrations of protein. The absorbance was read spectrophotometrically at 750 nm after 15 min reaction at 25° C. Crystalline bovine serum albumin was used as a standard.

Determination of methylglyoxal. MG in cell-free extracts was assayed enzymatically with glyoxalase I by a modified method as described in Bergmeyer (1986). In this assay, MG was quantitatively converted to *S*-D-lactoylglutathione by glyoxalase I in the presence of reduced glutathione. The increase in *S*-D-lactoylglutathione concentration was measured by the change in absorbance at 240 nm in a reaction mixture that contained the following in a final volume of 1.03 ml: K_2HPO_4–KH_2PO_4 buffer (pH 6.8), 97 mM; reduced glutathione, 2.5 mM; glyoxalase I, 1.4 kU/L; and 10 µl of samples containing MG. Absorbance at 240 nm was measured at 25° C against a blank, which included all reaction components except the glyoxalase I. The standard curve relating the MG concentration and the absorbance at 240 nm was linear over a range of MG concentrations from 0.1 to 5.0 mM. The absorption coefficient (E) for the product *S*-D-lactoylglutathione at 240 nm was 45.04. Thus, concentrations of MG in cell-free extracts and in fermentation broths were determined by

$$C = \text{Absorbance} \times 45.04(\text{mM}).$$

Extracellular MG was measured in the supernatant of fermentation broth from which cells were removed by centrifugation. Intracellular MG was determined in the cell-free extracts prepared by the procedures described above.

The intracellular MG concentration was measured from a known amount of fermentation broth. The MG contents in supernatant, C_s, and in disrupted cell concentrate, C_c, were measured by the method described above. The formula, which was used to calculate intracellular ethanol concentration by Novak *et al.* (1981) and Dasari *et al.* (1983), was modified and used to determine intracellular MG concentration C_i,

$$C_i = \frac{C_c \cdot V_c}{V_i},$$

where V_i is the volume of water within the cells, and V_c is the volume of the concentrated cell suspension. If x is defined as a mass fraction of water in a wet cell, V_i can be determined from the concentration of dry cell weight in broth (C_d),

$$V_i = \frac{C_d \cdot V_b \cdot x}{(1-x) \cdot 1000}\,(\text{liters}),$$

where V_b is the original volume of broth used for preparation of the MG sample. Assuming that 70% of *E. coli* protoplasm is water (Neidhardt and Umbarger, 1996), $x = 0.7$ is used here for calculation of intracellular MG. The concentration of dry cell weight, C_d, was calculated from optical density measured at 660 nm (OD_{660}) of broth using the following equation:

$$C_d = -0.0166 + 0.5183^* \, OD_{660}\ \text{g/L}\ (r = 0.9987).$$

This equation is a linear regression based on the means of three groups of independent measurements on the concentrations of dry cell weight (C_d) and the optical densities at 660 nm with the broths fermented by *E. coli* AG1.

Assay for glyoxalase I. Glyoxalase I was determined by a modified spectrophotometric assay delineated previously by Murata *et al.* (1986).The reaction mixture was in a final volume of 1.03 mL, which contained 97 mM phosphate buffer (pH 6.8), 2.5 mM reduced glutathione, and 5.0 mM MG. The reaction was initiated by the addition of 10 µl of homogeneous glyoxalase I or cell-free extracts to the above mixture. The enzymatic formation of *S*-D-lactoylglutathione was monitored at 240 nm until a steady maximum was achieved. The absorbance was measured against a blank, which was prepared in the same way as a sample

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except water instead of MG was added. The same value of E was used as in the MG assay. Activity was expressed as micromoles of MG converted per minute per gram of protein at 25° C.

Basic DNA techniques. Transformation of cells with plasmids was done following the procedures described by Maniatis *et al.* (1982). Plasmid DNA was prepared by the alkaline lysis of cells using a protocol of Promega Corp. (Madison, WI), followed by plasmid DNA purification using Qiagen-tip 20 (QIAGEN Inc., Chatsworth, CA). Agarose gel electrophoresis was done with a horizontal electrophoresis unit from Sigma Chemical (St. Louis, MO). Each DNA sample was diluted with an equal volume of loading solution from Sigma Chemical and loaded onto an agarose gel (1 % agarose was dissolved in a buffer of 0.045 M Tris–borate and 0.001 M EDTA). The gel was run at 76 V for 90 min.

Amplification of the P. putida glyoxalase I. An internal DNA fragment of the *P. putida* glyoxalase I gene was amplified by polymerase chain reaction (PCR) using a set of primers, 5′-GTCAAGGACATCGAGAAGTCG-3′ and 3′-GCATACTTGGTAGACCGGAAA-5′, which were designed from the known sequence (Rhee *et al.*, 1987). PCR was carried out in a 50-μl reaction mixture that contained 1.5 mM $MgCl_2$, 1 × PCR buffer (Perkin–Elmer), 0.2 mM dNTPs, 100 pmol of each primer, and 2.5 units of *Taq* DNA polymerase. Template DNA was provided by 2 μg of purified pTC13 or pTC9 plasmid. The PCR cycle consisted of 1 min at 94° C, 1 min at 55° C, and then 2 min at 72° C for 40 cycles. The expected product of 373 bp, corresponding to the middle section of the *P. putida* glyoxalase I gene, was excised from an agarose gel and purified using the QIAquick PCR purification kit from QIAGEN Inc.

Sequencing. The sequence of the 373-bp PCR product was determined using an automated DNA sequencer by the dideoxy chain termination method employing a sequencing kit (Perkin–Elmer Applied Biosystems ABI Prism). This sequence was compared with the published sequence of *P. putida* glyoxalase I (GenBank Accession No. D00342); the amplified sequence completely matched that of the corresponding section of the gene.

High-performance liquid chromatography analysis. 1,3-PD, glycerol, and other fermentation by-products such as lactic acid and acetic acid were analyzed by using a Bio-Rad HPLC system (Hercules, CA) fitted with a refractive index detector and a Bio-Rad Aminex HPX-87H organic acid column (Richmond, CA). The column temperature was maintained at 65° C. A 0.6 ml/min flow rate of 0.01 N sulfuric acid was utilized as the mobile phase. Samples were filtered through 0.45-μm Supor membranes (Gelman Sciences, Ann Arbor, MI); then, 50 μl was injected into the column.

RESULTS AND DISCUSSION

Examination of MG Accumulation during Anaerobic Glycerol Fermentation in E. coli AG1/pTC9

As described in previous studies (Skraly, 1997; Tong, 1992), high glycerol concentrations ($\geqslant 5$ g/L) inhibited cell growth and 1,3-PD production under anaerobic conditions in *E. coli* AG1 harboring the 1,3-PD production genes of *K. pneumoniae*. Accumulation of G3P was identified as one of the reasons for the inhibition (Zhu and Cameron, 1998). Here we analyzed whether the accumulation of MG might also contribute to inhibition of anaerobic glycerol fermentation with *E. coli* AG1/pTC9, a recombinant strain that is able to produce 1,3-PD under anaerobic conditions from glycerol. The MG levels in fermentation broth and in cells were assayed after 8 and 24 h of fermentation; the extracellular MG concentrations ranged from 0.34 to 0.47 mM, and the intracellular MG concentrations ranged from 1.49 to 1.63 mM, respectively. MG is known to halt proliferation of *E. coli* at concentrations of 0.2–1.0 mM (Együd and Szent-Györgyi, 1966; Rhee *et al.*, 1987) and is lethal at exogenous levels above 0.6 mM. The high intracellular and extracellular concentrations of MG observed here suggest that MG accumulation may have contributed to the inhibition of growth and 1,3-PD production at high glycerol concentrations.

Expression of the Glyoxalase I Gene of P. putida in E. coli AG1/pTC9

The glutathione-dependent glyoxalase I is the dominant detoxification enzyme for MG in *E. coli* grown aerobically (Maclean *et al.*, 1998). Before MG levels and its effects on anaerobic *E. coli* growth were characterized further, the gene encoding *P. putida* glyoxalase I with a high activity was introduced into the cells of *E. coli* AG1/pTC9.The source of this DNA was the 2.4-kb *Sal*I fragment of plasmid pGI423 (Rhee *et al.*, 1998). This fragment was ligated into the *Sal*I site of plasmid pTC9 containing the *K. pneumoniae dha* genes, and the ligated DNA was transferred into *E. coli* AG1 cells. The transformed cells were selected on LB-agar plates containing 50 μg/ml ampicillin. Ten ampicillin-resistant colonies were screened and one was found to contain a plasmid with the expected *Sal*I digestion pattern (15.9 and 2.4 kb). This plasmid was named pTC13. *E. coli* AG1/pTC9, which lacks the*P. putida* glyoxalase I gene, was defined as the control strain in this study.

The identity of the *P. putida* glyoxalase I gene was confirmed by PCR amplification of an internal 373-bp DNA fragment using glyoxalase I-specific primers and pTC13 as the template, as described. Sequence analysis of this PCR product corresponded exactly to that of the published sequence of the *P. putida* glyoxalase I gene (GenBank Accession No. D00342). Experiments measuring specific activities of glyoxalase I from cell-free extracts of *E. coli* AG1/pTC9 and *E. coli* AG1/pTC13 gave 11.2 ± 1.2 and 132.1 ± 11.5 μmol/min · g protein, respectively. The introduction of pTC13 into *E. coli* AG1 led to an approximately 12-fold increase in glyoxalase I specific activity over that in the control strain, *E. coli* AG1/pTC9. These results indicate that the 2.4-kb DNA insertion into pTC13 contains the *P. putida* glyoxalase I gene.

Because of its high specific activity, *P. putida* glyoxalase I has been introduced into microorganisms such as *E. coli* as well as into Chinese hamster ovary (CHO) cells to detoxify MG (Chaplen *et al.*, 1996; Maclean *et al.*, 1998; Rhee *et al.*, 1987). *E. coli* C600 cells transformed with the *P. putida* glyoxalase I gene showed a 155-fold increase in glyoxalase I activity over that in control cells (Rhee *et al.*, 1987). *E. coli* MJF274 cells, transformed with the *P. putida* glyoxalase I gene, exhibited a 430-fold elevation in glyoxalase I activity (Maclean *et al.*, 1998). Compared to the above two examples, the 12-fold increase in glyoxalase I activity seen here under anaerobic conditions in *E. coli* AG1/pTC13 was substantially lower. It is not clear what factors are responsible for this lower activity. The discrepancy in the expressed activity of glyoxalase I may be due to different *E. coli* strains used. Another reason may be that *P. putida* is an aerobic organism, so the activity of its glyoxalase I may be subject to some inhibition in an anaerobic environment.

Detoxification of MG in E. coli Containing the P. putida Glyoxalase I Gene

Detoxification of MG in *E. coli* by *P. putida* glyoxalase I was first characterized in experiments that were used to compare pTC13 and its predecessor, pTC9, in their ability to remove endogenous MG. Both intracellular and extracellular MG levels produced by *E. coli* AG1/pTC13 and *E. coli* AG1/pTC9 were measured at three different phosphate concentrations. Because MG synthase, a key enzyme responsible for MG synthesis in *E. coli*, is inhibited by inorganic phosphate (P_i) (Cooper, 1984; Hopper and Cooper, 1971), MG levels were expected to be inversely correlated to the $[P_i]$.The experiments were designed to investigate the role of glyoxalase I and also to examine MG levels at different $[P_i]$. Figure 2A shows that intracellular MG levels in the *P. putida* glyoxalase I-producing cells, *E. coli* AG1/pTC13, were consistently lower (35–43%) than those in the control strain, *E. coli* AG1/pTC9, at each P_i concentration tested. *E. coli* AG1/pTC13 also exhibited a 30% reduction in extracellular MG levels compared to the control (Fig. 2B). These decreases in both intracellular and extracellular MG concentrations are statistically significant ($P > 94\%$). Figures 2A and 2B also illustrate that, as expected,

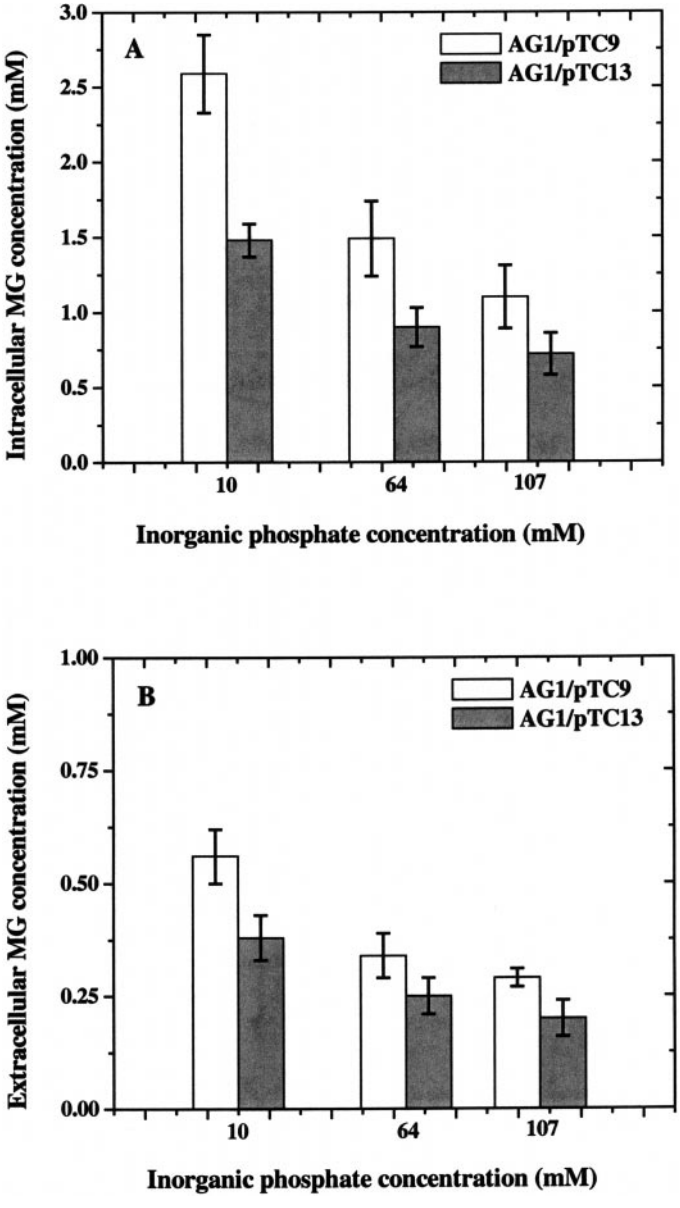

FIG. 2. Comparison of MG concentrations versus inorganic phosphate concentrations in *P. putida* glyoxalase I-producing strain *E. coli* AG1/pTC13 and in the control, *E. coli* AG1/pTC9. (A) Intracellular MG; (B) extracellular MG. Cells were grown anaerobically in a glycerol/glucose medium described under Materials and Methods. Samples were taken after 8 h of fermentation. Triplicate measurements were done. Values are means ± standard deviations, $n = 3$.

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both intracellular and extracellular MG levels were lower at the higher P_i concentrations, presumably because MG synthase is inhibited by P_i (Cooper, 1984; Hopper and Cooper, 1971).

Degradation of MG in *E. coli* by *P. putida* glyoxalase I was then characterized by comparing pTC13 and pTC9 in their ability to enable *E. coli* to grow on LB ampicillin plates containing increasing concentrations of MG. pGI423 was used as a positive control. *E. coli* AG1 was transformed with pTC9, pTC13, and pGI423, respectively, and then grown on LB-agar plates containing 0, 0.5, 1.0, and 2.0 mM MG. Results are shown in Fig. 3. About 80% of AG1/pTC13 and AG1/pGI423 colonies still survived at 0.5 mM MG. In contrast, only 2% of AG1/pTC9 colonies with wild-type glyoxalase I activity survived at this concentration. This result agrees well with that of Rhee *et al.* (1987), who reported that *E. coli* C600 cells are unable to grow on plates containing MG at a concentration above 0.6 mM.

The effect of exogenously added MG on cell growth was also determined under anaerobic conditions. *E. coli* AG1/pTC13 showed an increased resistance to MG. Without the addition of exogenous MG, *E. coli* AG1/pTC13 displayed a 56% increase in cell density (as measured by OD_{660}) compared to the control (Fig. 4A), suggesting that endogenously produced MG had a negative effect on cell growth.

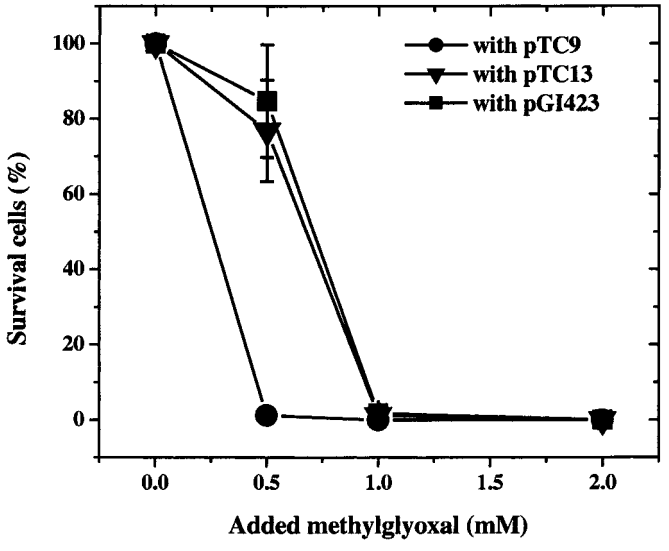

FIG. 3. Comparison of methylglyoxal resistance of *E. coli* cells. *E. coli* AG1 cells were transformed with pTC9 (●), pTC13 (▼), and pGI423 (■). Cells grew in LB medium for 12 h, then the same volume of cells of equal cell density was spread on LB plates containing 0–2.0 mM MG in triplicate. Colonies were counted (200–450 colonies on control plates) after 24 h of growth at 37° C. Cell survival is reported relative to that seen in the absence of added MG.

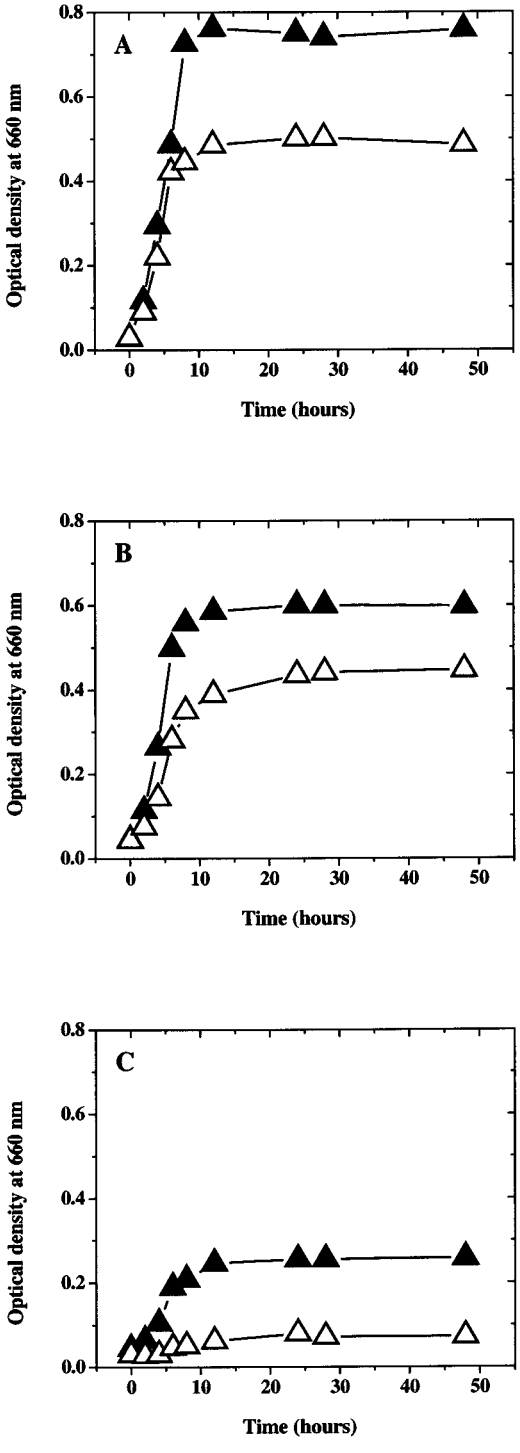

FIG. 4. Cell growth of *E. coli* AG1 transformed with pTC9 and pTC13 at different levels of exogenously added methylglyoxal. Cells were grown anaerobically in the medium described under Materials and Methods with the addition of methylglyoxal at 0.0 mM (A), 0.25 mM (B), and 0.5 mM (C). Symbols: AG1/pTC9 (△) and AG1/pTC13 (▲).

Moreover, both intracellular and extracellular levels of MG in *E. coli* AG1/pTC13 were lower than those in the control (Figs. 2A and 2B). Figure 4B shows that following addition of 0.25 mM MG to the culture medium, the OD_{660} of *E. coli* AG1/pTC13 was 35% higher than the control. The addition of 0.5 mM exogenous MG substantially inhibited growth of the control, *E. coli* AG1/pTC9 (Fig. 4C). As described above, *E. coli* AG1/pTC13 showed a 12-fold increase in the specific activity of glyoxalase I compared to *E. coli* AG1/pTC9. However, the OD_{660} of *E. coli* AG1/pTC13 was only 1.35–1.56 times higher than that of *E. coli* AG1/pTC9 when 0 to 0.25 mM exogenous MG was added. In other words, *E. coli* AG1/pTC13 gained 35–56% greater resistance to MG relative to AG1/pTC9 under these conditions. The discrepancy between the 12-fold increase in glyoxalase I activity and the 1.35- to 1.56-fold resistance indicates that the increased ability to resist MG is not linear with the elevation in glyoxalase I activity. A similar discrepancy between the gained resistance to MG and the increased activity in glyoxalase I was also observed by Ranganathan *et al.* (1995), who transfected the human glyoxalase I gene into murine cells and who attributed this discrepancy to the pharmacokinetics of inhibitors such as MG.

Finally, the effect of MG on 1,3-PD production was studied by comparing the final concentrations of 1,3-PD produced by *E. coli* AG1/pTC9 (the strain with only wild-type glyoxalase I) and by *E. coli* AG1/pTC13 (the strain with *P. putida* glyoxalase I) in parallel anaerobic fermentations with glycerol (5 g/L) and glucose (5 g/L). *E. coli* AG1/pTC9 produced 1.38 g/L of 1,3-PD, whereas *E. coli* AG1/pTC13 yielded 2.1 g/L of 1,3-PD. Compared to the control, *E. coli* AG1/pTC13 demonstrated 56% higher OD_{660} (Fig. 3) and 50% more 1,3-PD production. The similar gains in both OD_{660} and 1,3-PD production ability suggest that 1,3-PD production in *E. coli* is proportional to cell density, which is consistent with the result obtained by Skraly (1997). This result further indicates that MG accumulation is one of the reasons for inhibition of cell growth and 1,3-PD production.

CONCLUSION

In our study, we have shown that in anaerobic fermentation of glycerol/glucose by *E. coli* AG1/pTC9, endogenous MG inhibits both cell growth and 1,3-PD production. The expression of the glyoxalase I gene of *P. putida* in *E. coli* AG1/pTC9 resulted in statistically significant decreases in both intracellular and extracellular MG concentrations and provided better cell growth and improved 1,3-PD production. This study demonstrates an appraoch for reducing toxicity from metabolites by genetically modifying the biochemical pathways associated with a toxic compound and provides useful information for optimization of 1,3-PD production from both glycerol and sugars. In addition, it suggests a potential application of the glyoxalase I gene of *P. putida*, an aerobic organism, to detoxifying MG in anaerobic organisms.

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