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ORIGINAL PAPER

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The effect of carbon sources and lactate dehydrogenase deletion on 1,2-propanediol production in *Escherichia coli*

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Abstract In previous studies, we showed that cofactor manipulations can potentially be used as a tool in metabolic engineering. In this study, sugars similar to glucose, that can feed into glycolysis and pyruvate production, but with different oxidation states, were used as substrates. This provided a simple way of testing the effect of manipulating the NADH/NAD+ ratio or the availability of NADH on the metabolic patterns of *Escherichia coli* under anaerobic conditions and on the production of 1,2-propanediol (1,2-PD), which requires NADH for its synthesis. Production of 1,2-PD was achieved by overexpressing the two enzymes methylglyoxal synthase from *Clostridium acetobutylicum* and glycerol dehydrogenase from *E. coli*. In addition, the effect of eliminating a pathway competing for NADH by using a *ldh*$^-$ strain (without lactate dehydrogenase activity) on the production of 1,2-PD was investigated. The oxidation state of the carbon source significantly affected the yield of metabolites, such as ethanol, acetate and lactate. However, feeding a more reduced carbon source did not increase the yield of 1,2-PD. The production of 1,2-PD with glucose as the carbon source was improved by the incorporation of a *ldh*$^-$ mutation. The results of these experiments indicate that our current 1,2-PD production system is not limited by NADH, but rather by the pathways following the formation of methylglyoxal.

Keywords 1,2-Propanediol · *Escherichia coli* · Lactate dehydrogenase deletion

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Introduction

Biological redox reactions catalyzed by enzymes require a donor and/or acceptor of reducing equivalents in the form of electrons, hydrogen or oxygen atoms. Cofactor pairs that can be transformed reversibly between their reduced and oxidized states, nucleotide cofactors such as NADH/NAD+ and NADPH/NADP+ among others, serve this purpose very effectively in a living cell. The NADH/NAD+ cofactor pair, in particular, is very important in microbial catabolism, where a carbon source, such as glucose, is oxidized through a series of reactions utilizing NAD+ as a cofactor and produces reducing equivalents in the form of NADH. It is crucial for continued cell growth that NADH be oxidized and converted back to NAD+, thus achieving a redox balance. Under anaerobic growth and in the absence of an alternate oxidizing agent, this process occurs through fermentation, where NADH is used to reduce metabolic intermediates and regenerate NAD+ (Fig. 1) [10]. When sugars similar to glucose, but differing in their oxidation states, are used as substrates, *Escherichia coli* can redistribute its fermentation pattern to achieve a redox balance [1].

We showed how powerful cofactor manipulations can be, and have established them as an additional tool to achieve certain metabolic engineering goals. Some of the strategies we investigated include feeding carbon sources with different oxidation states [19], increasing intracellular NADH availability by an NADH regeneration strategy [7, 8], and increasing the total NAD levels by enhancing the NAD salvage pathway [9]. The main goals of our studies have been to investigate different external and genetic strategies of manipulating the total NAD levels and/or the intracellular availability of NADH and study their effect on the distribution of metabolites in *E. coli*, in particular, on the production of chemicals that require NADH for their synthesis, such as ethanol, succinate, and 1,2-propanediol (1,2-PD).

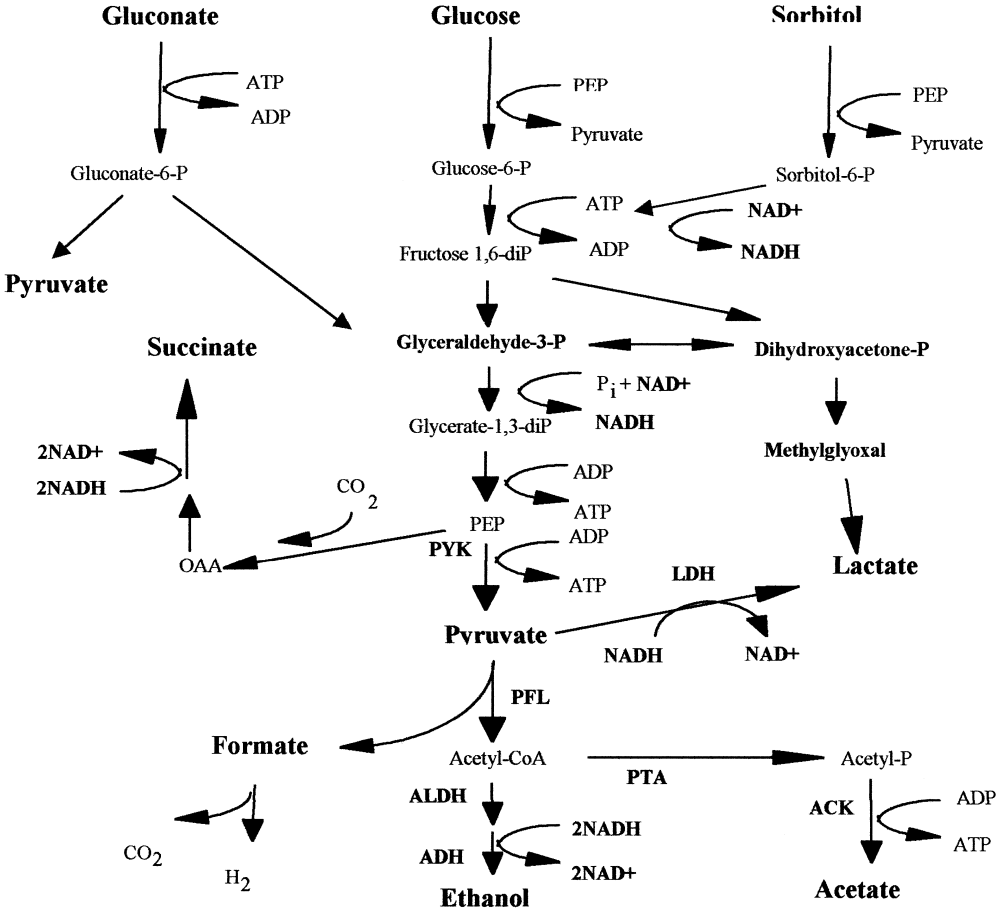

Fig. 1 Central anaerobic metabolic pathway of *Escherichia coli* showing generation of NADH, regeneration of NAD+, and the difference in oxidation between glucose, sorbitol and gluconate as they enter into glycolysis. Note differences in NADH production. Modified from [10]

In this paper we utilize the production of 1,2-PD as a model system to study the effect of changes in NADH on the production of a chemical that is synthesized through an engineered pathway. The production of 1,2-PD was chosen for this study because its biological synthesis depends on NADH, as shown in Fig. 2. The precursor dihydroxyacetone-P (DHAP) is a glycolytic intermediate. It can be converted to methylglyoxal (MG) by the enzyme methylglyoxal synthase (MGS), from which it is converted to D-lactate through the glyoxalase system [14]. This pathway is normally not very active due to the cytotoxicity of methylglyoxal [15]. However, MG can be converted to 1,2-PD by a series of two reductions. In this study, the production of 1,2-PD was achieved by overexpressing MGS from *Clostridium acetobutylicum* to increase the flux to MG and a glycerol dehydrogenase (GLDH) from *E. coli*.

1,2-PD is a major commodity chemical, with over one billion pounds produced annually in the USA, and has many industrial uses, such as unsaturated polyester resins, liquid laundry detergents, pharmaceuticals, cosmetics, antifreeze and deicers [12]. Work on microbial production of 1,2-propanediol has been reviewed in recent years [5, 12]. The production of 1,2-PD in different strains, such as *Clostridium sphenoides*, *Thermoanaerobacterium thermosaccharolyticum*, as well as recombinant strains of *E. coli* and *Saccharomyces cerevisiae*, has been extensively studied by Cameron and coworkers [2, 3, 4, 11, 12, 16].

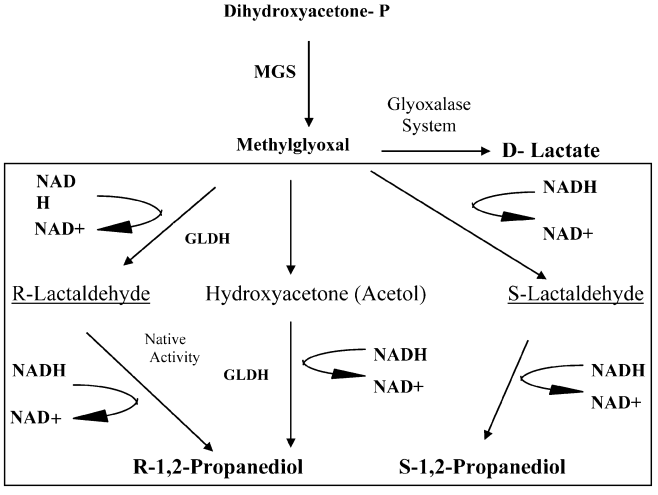

Fig. 2 Pathway to 1,2-propanediol (1,2-PD) in *E. coli* [2]. Fluxes inside the box are to be incorporated. MGS and GLDH are being overexpressed in this study. It is believed that our engineered strain follows the pathway on the left to produce 1,2-PD. In our engineered pathway, DHAP is converted to MG by MGS, MG is reduced to R-lactaldehyde by GLDH and a native *E. coli* activity reduces it to 1,2-PD. *MGS* Methylglyoxal synthase, *GLDH* glycerol dehydrogenase

In the present study, glucose (oxidation state = 0), sorbitol (oxidation state = –1), and gluconate (oxidation state = +1) were used as carbon sources. Figure 1 shows the central anaerobic metabolic pathway of *E. coli* with glucose, sorbitol or gluconate as carbon sources. As the figure shows, sorbitol will produce more reducing equivalents in the form of NADH than glucose. On the other hand, gluconate produces less NADH than glucose, since half of every molecule goes directly to pyruvate, skipping the NADH-producing step in glycolysis. This provided a simple way of testing the effect of manipulating the NADH/NAD+ ratio on the metabolic patterns of *E. coli* under anaerobic conditions and on the production of 1,2-propanediol, which requires NADH. In addition, the effect of eliminating a pathway that competes for NADH utilization by using a *ldh*$^-$ strain without lactate dehydrogenase activity on the production of 1,2-PD was investigated.

Materials and methods

Strains and plasmids

Table 1 lists the plasmids and *E. coli* strains used.

Construction of plasmid pSBPD

The construction of plasmid pSBPD is outlined below. Plasmid pSBPD contains the glycerol dehydrogenase (*gldA*) gene from *E. coli* and the methylglyoxal synthase (*mgs*) gene from *C. acetobutylicum* in the backbone of pBCSK+ (Cm^R). It was necessary to construct an intermediate plasmid (pSBPDI) using pUC18 as a cloning vector to obtain the appropriate restriction sites. The plasmid pGLDH was digested with *Eco*RI/*Xba*I, pMGS2 was digested with *Xba*I/*Pst*I, and pUC18 with *Eco*RI/*Pst*I. The products of these restriction digestions were ligated together and transformed into *E. coli* strain DH10B. Minipreps of white colonies from Ap/IPTG/Xgal plates were digested with *Eco*RI and analyzed by agarose gel electrophoresis to select the right clones. The intermediate plasmid and pBCSK+ were then digested with *Eco*RI/*Xho*I and ligated. The ligation product was transformed into *E. coli* strain DH10B. The desired clone was identified among the white colonies on Cm/IPTG/Xgal plates by agarose gel electrophoresis of miniprep DNA digested with *Eco*RI/*Xho*I. The resulting pSBPD plasmid contains the *gldA* gene from *E. coli* and the *mgs* gene from *C. acetobutylicum* in the backbone of pBCSK+ (Cm^R). This plasmid, as well as the pBCSK+ (as a control), was transformed into GJT001 and YBS131 for the 1,2-propanediol production studies. The newly constructed plasmid, pSBPD, was characterized by measuring the GLDH and MGS activity of cells harboring the plasmid.

Medium

The production medium consisted of M9 medium (6 g/l Na_2HPO_4, 3 g/l KH_2 PO_4, 1 g/l NH_4Cl, 0.5 g/l NaCl, 2 mM $MgSO_4$) supplemented with 13.3 g/l $NaHCO_3$, 5 g/l yeast extract [2], different amounts of carbon source (glucose, sorbitol, or gluconate) and 34 mg/l chloramphenicol and/or 25 mg/l tetracycline.

Experimental procedures

The 1,2-propanediol production experiments were performed using 40 ml (anaerobic experiments) or 45 ml (microaerobic experiments) glass vials with PTFE/silicone rubber septa on open-top caps. Each vial was filled with 35 ml of production medium and inoculated with 100 µl of a 5 ml LB overnight culture. For the anaerobic experiments, 6 ml of air was removed with a syringe from the headspace to ensure anaerobic conditions. For the microaerobic experiments, initial removal of air from the headspace was omitted, making them aerobic initially and turning anaerobic eventually. This was done to allow better growth in the presence of oxygen during the initial stage. The cultures were grown in a shaker at 37°C and 250 rpm. A sample of the initial medium was saved for analysis and samples were withdrawn with a syringe for analysis at 24-h intervals.

Analytical techniques

Cell density (OD) was measured at 600 nm in a spectrophotometer. Fermentation samples were centrifuged for 5 min in a microcentrifuge. The supernatant was filtered through a 0.45-micron syringe filter and stored chilled for HPLC analysis. The fermentation products, as well as the carbon sources and 1,2-propanediol, were quantified using an HPLC system (Thermo Separation Products) equipped with a cation-exchange column (HPX-87H, BioRad Laboratories) and a differential refractive index detector. A mobile phase of 2.5 mM H_2SO_4 at a 0.6 ml/min flow rate was used and the column was operated at 55°C.

MGS and GLDH activity assays

GJT001(pSBPD) and GJT001(pBCSK) were grown overnight in 40 ml of production medium supplemented with 20 g/l glucose

Table 1 Strains and plasmids used in this study

	Significant genotype	References
Strains		
GJT001	Spontaneous cadR mutant of MC4100, Sm^R	[20]
DH10B	Cloning host	[20]
YBS131	GJT001 *ldhA*::Tn10, Tc^R	[21]
Plasmids		
pUC18	Control, cloning vector, Ap^R	Stratagene
pBCSK+	Control, cloning vector, Cm^R	Stratagene
pGLDH	*gldA* plasmid, Km^R	[17]
pMGS2	*mgs* plasmid, Ap^R	[17]
pSBPDI	*gldA* and *mgs* plasmid, constructed from pUC18, pGLDH and pMGS2, Ap^R	This study
pSBPD	*gldA* and *mgs* plasmid, constructed from pBCSK+, pGLDH and pMGS2, Cm^R	This study

and 34 mg/l chloramphenicol. Cultures were inoculated with a colony from a plate and grown in a shaker at 37°C and 250 rpm. Cells were harvested by centrifugation of 20 ml of culture at 4,000 *g* and 4°C for 10 min. The pellet was suspended in 10 ml of 50 mM imidazole–HCl buffer (pH = 7.0) and centrifuged as described above. The cells were resuspended in 10 ml of 50 mM imidazole–HCl buffer and sonicated for 6 min in an ice bath. Sonicated cells were centrifuged at 1,500 *g* and 4°C for 60 min to remove cell debris and reduce the NAD background.

Glycerol dehydrogenase activity was assayed at 37°C by adding 100 µl of cell extract to 1 ml of a reaction mixture containing 0.495 mg/ml NADH and 126 mM acetol (hydroxyacetone) in imidazole–HCl buffer and measuring the decrease in absorbance of NADH at 340 nm. One unit was defined as the amount of enzyme that oxidized 1 µmol NADH per minute at 37°C [2].

Methylglyoxal synthase activity was determined using a colorimetric assay [13], which consisted of a reaction step that produced methylglyoxal followed by a detection step. Two test tubes containing a reaction mixture consisting of 0.4 ml of 50 mM imidazole–HCl buffer (pH = 7.0), 25 µl of 15 mM DHAP and 50 µl distilled water were incubated in a water bath at 30°C. The assay was initiated by adding 25 µl of crude enzyme extract to one of the reaction mixture tubes. The other tube was used for the control assay, in which the crude extract was substituted by 25 µl of imidazole buffer. Immediately after 2.5 min, 5 min and 10 min at 30°C, 0.1 ml aliquots were removed from the reaction mixtures and added to the appropriate detection mixture tubes. The detection mixture contained 0.9 ml distilled water and 0.33 ml 0.1% 2,4-dinitrophenylhydrazine dissolved in 2 N HCl per tube. After incubating the detection mixtures with the aliquots at 30°C for 15 min, 1.67 ml of 10% NaOH was added to each tube to produce a purple color. This mixture was incubated at room temperature for 15 min and the absorbance at 550 nm was recorded. One unit of MGS is defined as that amount of enzyme catalyzing the formation of 1 µmol of methylglyoxal per minute at 30°C. (In this system 1 µmol of methylglyoxal has $A_{550nm} = 16.4$.) Total protein concentration in cell extracts was measured by Lowry's method (Sigma Kit) using bovine serum albumin as standard.

Results and discussion

This study investigates the effect of manipulating NADH availability on the production of an NADH-dependent chemical, 1,2-propanediol. The involvement of NADH in the synthesis of 1,2-PD is illustrated in Fig. 2.

Plasmid characterization

The production of 1,2-PD was achieved by overexpressing the enzyme MGS, to increase the flux to MG, and GLDH. For this purpose, a plasmid containing the glycerol dehydrogenase gene (*gldA*) from *E. coli* and the methylglyoxal synthase gene (*mgs*) from *C. acetobutylicum* was constructed and characterized by measuring the activity of these enzymes in cells transformed with this plasmid. The results of the plasmid characterization are shown in Table 2. Cells bearing plasmid pSBPD showed a 51-fold increase in GLDH activity and a 535-fold increase in MGS activity relative to cells containing the control plasmid pBCSK+.

Table 2 Plasmid characterization. Results of GLDH and MGS activity assays. GLDH: 1U = amount of enzyme catalyzing the oxidation of 1 µmol of NADH per minute at 37°C. MGS: 1U = amount of enzyme catalyzing the formation of 1 µmol of methylglyoxal per minute at 30°C

Enzyme	Activity (U/mg)		
	pBCSK+	pSBPD	Increase (fold)
GLDH	0.003	0.143	51
MGS	0.004	2.272	535

Carbon source experiment

We demonstrated that carbon sources similar to glucose but with different oxidation states can be used to manipulate the NADH/NAD+ ratio or NADH availability and can serve as a simple means of determining whether a particular pathway is limited by NADH [19]. For this reason, experiments were performed with glucose, sorbitol or gluconate as carbon sources in a 1,2-propanediol production system under anaerobic and microaerobic conditions.

The effect of initial glucose concentration on 1,2-PD production under anaerobic conditions after 48 h of culture was investigated (data not shown) [6]. A maximum was observed at about 100 mM initial glucose concentration. The same trend was observed after 24 and 72 h of culture (data not shown) [6]. For this reason, the rest of this article presents only the data from experiments with an initial carbon source concentration of 100 mM. In addition, differences in carbon source consumption with 100 mM initial concentration under anaerobic conditions for the three carbon sources were studied (data not shown) [6]. It was observed that gluconate uptake experiences a longer lag time after which its uptake surpasses the uptake of the other two carbon sources. On the other hand, the utilization of sorbitol is relatively similar to that of glucose, although slightly lower.

Figure 3 shows the yields of fermentation products in moles produced per mole of carbon source consumed after 48 h of anaerobic culture. From the carbon source experiment, it was observed that the oxidation state of the carbon source significantly affected the yield of metabolites, such as ethanol, acetate, and lactate. The use of a more reduced carbon source (sorbitol) led to a significant increase in the yield of ethanol, a reduced product, accompanied by a significant decrease in the yield of acetate, a more oxidized product that does not require NADH for its synthesis. On the other hand, the use of gluconate as a carbon source provoked a significant increase in the yield of acetate and a decrease in the ethanol yield. In other words, feeding a more reduced carbon source (sorbitol) provides a more reduced environment as evidenced by the higher ethanol to acetate (Et/Ac) ratio (Fig. 4). The Et/Ac ratio was highest for sorbitol and lowest for gluconate compared

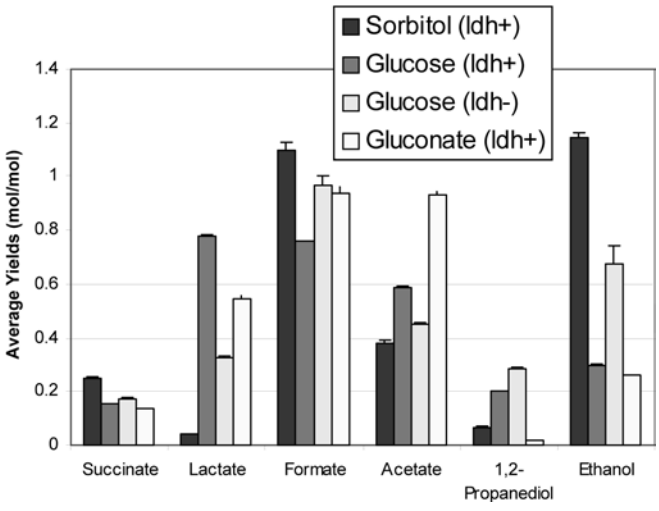

Fig. 3 Yields of metabolites from anaerobic cultures with different carbon sources for the 1,2-PD producing strains. An initial carbon source concentration of 100 mM was used. Results shown are yields of fermentation products (average of triplicates) in moles produced/mole of carbon source consumed after 48 h of culture. *ldh*$^+$ refers to strain GJT001(pSBPD) and *ldh*$^-$ refers to strain YBS131 (pSBPD)

with glucose, which normally produces equimolar quantities of these two products. This is in accordance with the oxidation state of the carbon sources used and with our previous report [19] in which a higher Et/Ac ratio was shown to serve as an indirect indicator of a higher NADH/NAD+ ratio.

The level of lactate, which consumes 1 NADH through lactate dehydrogenase, was significantly reduced when sorbitol was the carbon source and was relatively high with glucose and gluconate (Fig. 3). The results found for sorbitol can be explained by a preference to get rid of the excess reducing power provided by this carbon source through production of ethanol (2 NADH) over the production of lactate (1 NADH), since ethanol consumes more reducing power than lactate. In a similar way, when gluconate is used as the carbon source, the cells regenerate NAD+ through the less expensive of these two pathways in terms of consumption of reducing equivalents. Therefore, it produces more lactate than ethanol. This is also in agreement with our previous findings under anaerobic chemostat conditions [19].

In terms of 1,2-PD production, as expected, gluconate had the lowest 1,2-PD yield. This is due in part to the more oxidized environment provided by this carbon source and to the fact that half of it goes directly to pyruvate, not producing DHAP, an intermediate in 1,2-PD synthesis. Although it was observed from the ethanol/acetate ratios (Fig. 4) that a more reduced environment was provided by feeding a more reduced carbon source, unexpectedly sorbitol did not increase the yield of 1,2-PD. Cells were getting rid of the excess reducing power by increasing the production of ethanol, which consumes 2 NADH. The ethanol pathway may be more competitive for this purpose than the 1,2-PD pathway (GLDH) and even the LDH. In addition, manipulating the NADH/NAD+ ratio by feeding a more reduced carbon source (sorbitol) may produce a high excess of reducing power that can put the cells under stress and force them to get rid of it in the most effective way. The formation of ethanol seems to provide such a means. This idea is supported by the finding that NADH induces expression of alcohol dehydrogenase (*adhE*), one of the enzymes involved in ethanol production [18].

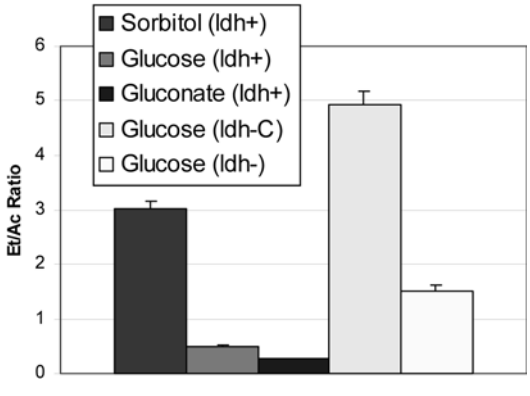

Fig. 4 Comparison of the ethanol to acetate ratios. Strains: *ldh*+ = GJT001 (pSBPD); *ldh*-C = YBS131(pBCSK); *ldh*– = YBS131(pSBPD). The three carbon sources were used at 100 mM initial concentration under anaerobic conditions

Studies with a strain deficient in lactate dehydrogenase

Based on the high lactate levels produced from glucose (Fig. 3) (highest 1,2-PD yield), and the fact that it consumes NADH, it was decided to eliminate that NADH and carbon competing pathway by using a *ldh*$^-$ strain. This approach should increase the reducing power available while still using glucose as the carbon source.

The results on the anaerobic production of 1,2-PD with glucose as the carbon source are summarized in Table 3 for the *ldh*$^-$ strain versus GJT001. Figure 5 compares the production of 1,2-PD after 48 h experimental conditions under anaerobic and microaerobic growth.

The final concentration and the yield of 1,2-PD were higher for the *ldh*$^-$ strain YBS131 (pSBPD) than the parental strain GJT001 (pSBPD) under both experimental conditions (Table 3, Fig. 5). The yield of 1,2-PD increased by 43% for YBS131 (pSBPD) relative to GJT001 (pSBPD) under anaerobic conditions, and by 67% under microaerobic conditions. The production of 1,2-PD for the same strain showed no significant difference between anaerobic and microaerobic conditions (Fig. 5).

Feeding a more reduced carbon source (sorbitol) and eliminating a NADH competing pathway (*ldh*), both provided a more reduced environment, as evidenced by their Et/Ac ratios (both higher than for the *ldh*$^+$ strain

Table 3 1,2-Propanediol yields from 24 and 48 h anaerobic cultures of GJT001 (pSBPD) and YBS131 (pSBPD) (*ldh*⁻ strain) with glucose as the carbon source

Ineutration time (h)		Initial glucose concentration (mM)	Glucose consumed (mM)	PD concentration (mM)	PD (g/l)	PD % yield (mol/mol)	PD yield (g/g)
24	GJT001 (pSBPD)	45.91	45.91	4.90	0.37	10.65	0.05
		101.68	48.19	7.86	0.60	16.31	0.07
		196.17	53.63	7.00	0.53	13.01	0.05
	YBS131 (pSBPD)	98.88	45.60	10.80	0.82	23.70	0.10
48	GJT001 (pSBPD)	45.91	45.91	4.88	0.37	10.62	0.04
		101.68	71.15	14.18	1.08	19.94	0.08
		196.17	76.17	11.79	0.90	15.70	0.07
	YBS131 (pSBPD)	98.88	59.76	17.07	1.30	28.56	0.12

with glucose as the carbon source). However, their effect on production of 1,2-PD was different. The *ldh*⁻ strain with glucose as the carbon source produced 1,2-PD at higher levels than the *ldh*⁺ strain with sorbitol as the carbon source (Fig. 5).

The highest 1,2-PD yields achieved in this study (0.13 and 0.12 g/g) were obtained with YBS131 (pSBPD) (*ldh*⁻ strain) with glucose as the carbon source under microaerobic and anaerobic conditions, respectively. This value is consistent with a literature report in which a similar system was used but utilizing both *E. coli mgs* and *gldhA* [3].

Experiments with the *ldh*⁻ strain provide important information about the system. The *ldh*⁻ strain with *mgs* and *gldhA* overexpressed showed an increase in lactate production compared with the *ldh*⁻ control strain (Fig. 6) and a decrease in the Et/Ac ratio. This decrease in the Et/Ac ratio suggests that part of this observed difference in reducing power is used for 1,2-PD production. The increase in the lactate level indicates that some methylglyoxal produced by the very active MGS from *C. acetobutylicum* is being converted to lactate, possibly through the glyoxalase pathway (see Fig. 1), which serves as a mechanism to detoxify methylglyoxal. The lactate yield for YBS131 (pSBPD) was 0.191 g/g of glucose, 80% higher than that reported in the literature (0.106 g/g of glucose) for a similar system, but over-

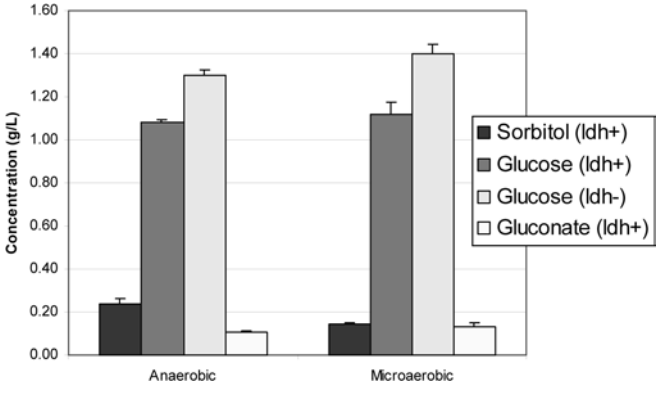

Fig. 5 Production of 1,2-PD after 48 h of culture for GJT001 derivatives with 100 mM initial concentration of various carbon sources under anaerobic and microaerobic growth

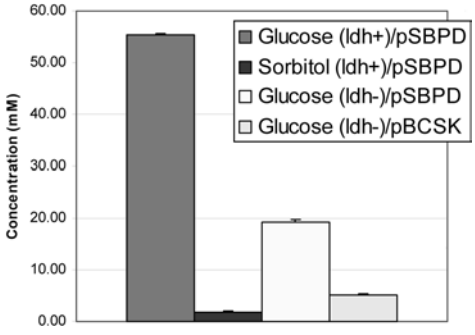

Fig. 6 Lactate concentration from anaerobic cultures of the GJT001 derivatives with 100 mM initial concentration of sorbitol or glucose after 48 h of growth

expressing the MGS from *E. coli* instead [3]. This implies that the MGS from *C. acetobutylicum* is more active than that of *E. coli*. However, the full potential of this enzyme for the production of 1,2-PD is not exploited in the current system due to the limitation in the pathway following the production of MG, leading to a higher overflow to lactate through the glyoxalase pathway. This observation suggests that the production of 1,2-PD can be further improved by optimizing the steps in the pathway subsequent to the production of MG. After this is achieved, the use of a glyoxalase mutant strain might improve production. However, the use of a glyoxalase mutant strain with the current system may cause toxic levels of MG to accumulate due to the high activity of the *C. acetobutylicum* MGS.

These results, together with the results of the carbon source experiment, indicate that the current 1,2-PD production system is not limited by NADH. The methods of NADH/NAD+ manipulation presented in this paper may increase the synthesis of a product in an NADH-limited system. Therefore, it may be possible to further increase production of 1,2-PD using the methods investigated in this study after the bottleneck in the current pathway is removed, for example, by overexpressing the whole pathway [3].

Additional attempts were made to improve 1,2-PD production by eliminating competing pathways such as *pfl* and *pfl*/*ldh*, but these proved unsuccessful. A strain with a *pfl*⁻ mutation was constructed and transformed with a 1,2-PD-producing plasmid, but it produced

mostly high lactate levels anaerobically and negligible amounts of 1,2-PD. A strain incorporating both a *pfl*⁻ and a *ldh*⁻ mutation was also constructed, transformed with a 1,2-PD-producing plasmid, and tested, but presented poor growth under anaerobic conditions as well as negligible amounts of 1,2-PD (data not shown) [6].

Conclusions

Feeding a more reduced carbon source did not increase the yield of 1,2-PD. Instead, the additional reducing power provided was directed toward production of ethanol. The 1,2-PD production with glucose as the carbon source was improved by incorporation of the *ldh*⁻ mutation in the host. The highest 1,2-PD yields achieved (0.13 and 0.12 g/g) were obtained with YBS131 (pSBPD) (*ldh*⁻ strain) with glucose as the carbon source under microaerobic and anaerobic conditions, respectively. The results obtained so far indicate that our current 1,2-PD production system is not limited by NADH. Therefore, it is possible to further improve our current 1,2-PD pathway and increase the production of 1,2-PD through other metabolic engineering approaches.

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