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MINI-REVIEW

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Microbial formation, biotechnological production and applications of 1,2-propanediol

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Abstract This short review covers metabolic pathways, genetics and metabolic engineering of 1,2-propanediol formation in microbes. 1,2-Propanediol production by bacteria and yeasts has been known for many years and two general pathways are recognized. One involves the metabolism of deoxyhexoses, where lactaldehyde is formed during the glycolytic reactions and is then reduced to 1,2-propanediol. The second pathway derives from the formation of methylglyoxal from dihydroxyacetonephosphate and its subsequent reduction to 1,2-propanediol. The enzymes involved in the reduction of methylglyoxal can generate isomers of lactaldehyde or acetol, which can be further reduced by specific reductases, giving chiral 1,2-propanediol as the product. The stereospecificity of the enzymes catalyzing the two reduction steps is important in deriving a complete pathway. Through genetic engineering, appropriate combinations of enzymes have been brought together in *Escherichia coli* and yeast to generate 1,2-propanediol from glucose. The optimization of these strains may yield microbial processes for the production of this widely used chemical.

General and historical outline

Two types of propanediol can be made by bacteria. 1,3-Propanediol, an intermediate in the synthesis of polyurethane and polyesters, can be prepared by microbial fermentation by the reduction of glycerol (Tong et al. 1991; Skraly et al. 1998); and a microbial process for forming 1,3-propanediol is being advanced by DuPont. This area has been reviewed recently (Biebl et al. 1999).

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1,2-Propanediol (propylene glycol) can be produced by a variety of bacteria. Early studies on *Clostridium thermobutyricum* (Enebo 1954), yeasts (Suzuki and Onishi 1968), *Escherichia coli* (Cooper and Anderson 1970; Cooper 1975), and other organisms including *Bacteroides ruminicola* (Turner and Roberton 1979) have demonstrated 1,2-propanediol formation. The biosynthesis of 1,2-propanediol is through two main routes, one in which deoxy sugars are used as the carbon source (and in this route the key intermediate, lactaldehyde, is formed directly from a glycolytic reaction) while in the other route conversion of the glycolytic intermediate, dihydroxyacetone phosphate, to methylglyoxal is the crucial branch. Subsequent reduction of methylglyoxal can yield 1,2-propanediol. Useful reviews related to this topic are given in Lin (1996), Cameron et al. (1998), and Altaras and Cameron (1999). Recent studies on the utilization of propanediol (Bobik et al. 1999) and the introduction of the *dha* operon into *E. coli* (Sprenger et al. 1989) and applications in the bioremediation of glycols also have attracted interest (Shieh et al. 1998).

1,2-Propanediol from an economic perspective

About 0.5 billion kg of racemic 1,2-propanediol (propylene glycol) derived from petroleum are used industrially in the United States annually (Cameron et al. 1998). About 50 million kg were used in food products, although recent developments (Bauer et al. 1992) have led to the removal of propylene glycol from GRAS status for use in cat food (Federal Register 1996). 1,2-Propanediol also has a valuable use as a less toxic alternative in antifreeze and as a de-icer. The compound is also used as a feedstock in the preparation of polyester resins for film and fiber manufacture. The pure stereoisomers produced by microbial fermentation would have additional value as chiral starting materials for the synthesis of specialty chemicals, such as optically active propylene oxide and polymers; and they may be useful in the manufacture of chiral pharmaceutical products.

The chemical route to 1,2-propanediol is through the hydration of propylene oxide (Martin and Murphy 1994). The precursor compound, propylene oxide, is formed chemically either by the chlorohydrin process or by the hydroperoxide process (Trent 1996). Both of these processes have some disadvantages. The chlorohydrin process consumes chlorine and caustic soda in stoichiometric quantities and thus has costs associated with hazardous chemical use. The hydroperoxide method also produces either *t*-butyl alcohol or phenylethanol as co-products, so the overall process must consider the value and use of these co-products. While phenylethanol can serve as a precursor of styrene, *t*-butyl alcohol has a less broad market.

Natural formation of 1,2-propanediol by microbes

The formation of 1,2-propanediol from the deoxy sugars (methyl pentoses), rhamnose and fucose, is widely known and occurs in many bacteria. The pathway has been characterized in *E. coli* and *Salmonella typhimurium* (Badia et al. 1985) and is reviewed by Lin (1996; Fig. 1). The system has been most thoroughly studied in *E. coli*. Upon catabolism of L-rhamnose to L-rhamnulose-1-phosphate, the phosphorylated sugar is cleaved by an aldolase, RhaD, to produce dihydroxyacetone phosphate and S-lactaldehyde (L-lactaldehyde; Badia et al. 1985). Metabolism of fucose can also generate lactaldehyde (Boronat and Aguilar 1979). Fucose is similarly metabolized upon entry into the cell. It is isomerized to L-fuculose and then phosphorylated by L-fuculose kinase to L-fuculose-1-phosphate. The aldolase encoded by *fucA* cleaves the molecule into L-lactaldehyde and dihydroxyacetone phosphate. Under anaerobic conditions, the lactaldehyde is reduced to S(+)-1,2-propanediol (L-1,2-propanediol) by a NAD-oxidoreductase, FucO. The propanediol product seems to be excreted by facilitated diffusion (Hacking et al. 1978). While *fucO* can be induced under aerobic and anaerobic conditions, it is normally active only anaerobically (Boronat and Aguilar 1981; Chen and Lin 1984). *E. coli* mutants that constitutively express *fucO* have been isolated (Wu 1976b; Boronat and Aguilar 1981). Some mutants have also been isolated that can utilize 1,2-propanediol as a carbon source (Sridhara et al. 1969; Cocks et al. 1974; Wu 1976a; Hacking and Lin 1977). A variety of solventogenic clostridial strains can produce 1,2-propanediol by this pathway from deoxy sugars (Forsberg et al. 1987). This route is not commercially feasible, due to the high cost of fucose and rhamnose.

Another natural route to 1,2-propanediol is found in *Thermoanaerobacterium thermosaccharolyticum* (formerly *C. thermosaccharolyticum*) and *C. sphenoides*, which are able to convert glucose to R(−)-1,2-propanediol [D(−)-1,2-propanediol] via the methylglyoxal pathway (Tran-Din and Gottschalk 1985; Cameron and Cooney 1986; Fig. 2). After formation of the intermediate, methylglyoxal, by methylglyoxal synthase, it

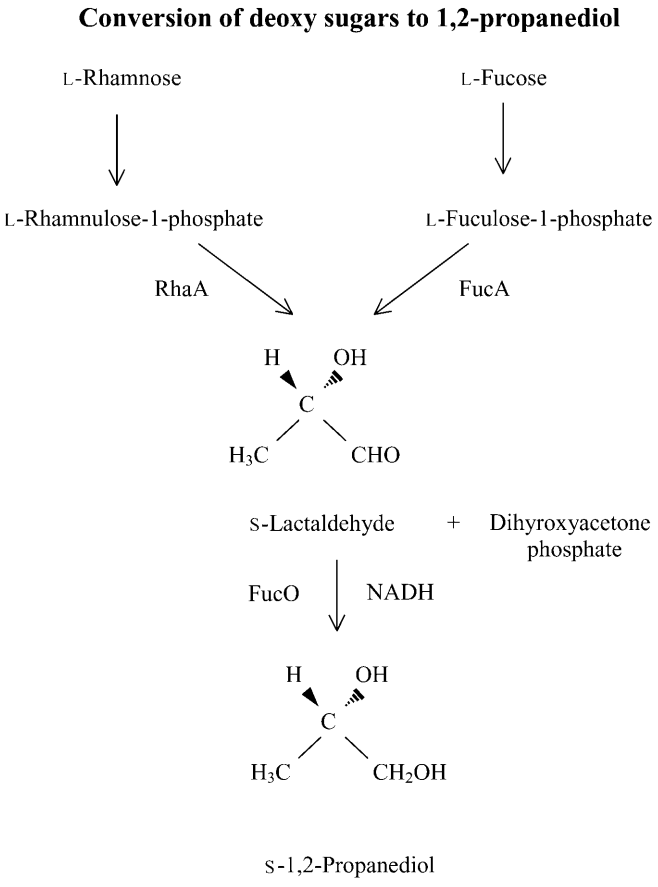

Fig. 1 The conversion of deoxy sugars to 1,2-propanediol. The pathway and gene products active in specific steps in *Escherichia coli* are shown

can be reduced to the chiral R(−)-1,2-propanediol by a bacterial aldose reductase and glycerol dehydrogenase (Altaras and Cameron 1999). *C. sphenoides* can form R(−)-1,2-propanediol from rhamnose and fucose and can also form the compound from glucose, fructose, cellobiose, and some other sugars (Tran-Din and Gottschalk 1985). A concentration of 20.8 mM 1,2-propanediol was formed after 96 h, about one-quarter of the concentration of ethanol formed. In order to produce 1,2-propanediol from glucose, *C. sphenoides* required culture under phosphate limitation (less than 0.08 mM). The methylglyoxal synthase from this organism was strongly inhibited by phosphate ($K_i = 1.9$ mM), although the methylglyoxal reductase enzyme activity was not inhibited by phosphate. The formation of methylglyoxal synthase was not repressed upon culturing in phosphate medium, unlike the situation with the methylglyoxal reductase and propanediol dehydrogenase activities, which were several-fold lower in cell extracts from cultures grown with high phosphate. Analyses showed a cell extract of *C. sphenoides* converted 1,2-propanediol and NAD^+ to lactaldehyde.

Three strains of *T. thermosaccharolyticum* were found to produce R(−)-1,2-propanediol from glucose, xylose, mannose, and cellobiose, but not maltose, fructose, or

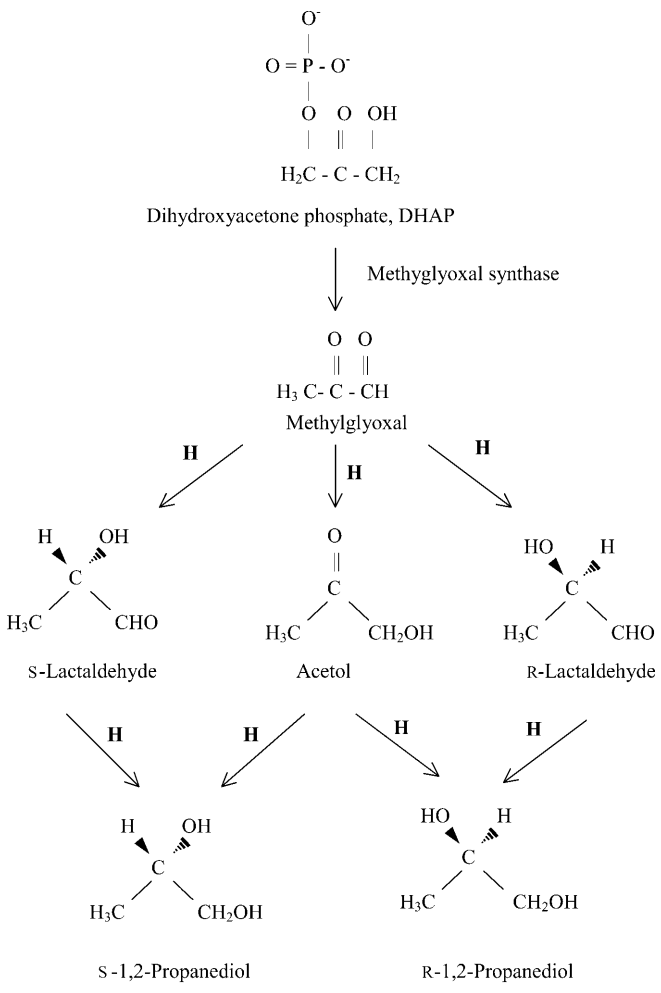

Fig. 2 The conversion of methylglyoxal to 1,2-propanediol. Various possible routes are shown. The three possible initial reduction products are indicated. The *bold H* indicates participation of a reduction step. The biological reductant is usually NADH or NADPH

lactose (Cameron and Cooney 1986). Conversion from glucose was as high as 0.27 g propanediol g^{-1} glucose. The intermediate in the reduction was acetol. The production of propanediol was not affected by phosphate concentration; and medium containing 113 mM phosphate could be used. A conversion of glucose to acetate, 1,2-propanediol, and CO_2 would result in a theoretical yield of 0.42 g propanedeiol g^{-1} glucose. A suggestion to improve the yield by reducing lactate and ethanol production was discussed (Cameron and Cooney 1986). It was pointed out that if additional reducing power (e.g., H_2) could be used, the ratio of 1,2-propanediol to acetate could be increased, improving the possible yield to 0.56 g g^{-1} glucose.

Low levels of 1,2-propanediol have been detected in several industrial *Saccharomyces cerevisiae* fermentations (Dowd et al. 1993, 1994). However, all these fermentations involved undefined media which might contain one of the intermediates in the pathway, which could be converted to 1,2-propanediol. Hence, there is no direct evidence that *S. cerevisiae* produces 1,2-propanediol from the fermentation of sugar. Nevertheless, these observations do suggest that *S. cerevisiae* contains some of the basic 1,2-propanediol enzymes for production, and it may be used as a potential host system to produce 1,2-propanediol. In this regard, enzymes capable of reducing methylglyoxal have been isolated from yeast (Table 1).

Enzymes involved in 1,2-propanediol synthesis

methylglyoxal synthase

Although methylglyoxal formation was detected in *Pseudomonas saccharophila* treated with iodoacetate (Entner and Doudoroff 1952), no enzymatic reaction was identified. Early reports on the formation of methylglyoxal in *E. coli* suggested its formation from glyceraldehyde 3-phosphate. Purification of the enzyme from *E. coli* and other organisms showed that dihydroxyacetone phosphate was the substrate and the enzyme was very sensitive to phosphate (Cooper and Anderson 1970; Freedberg et al. 1971; Hopper and Cooper 1971, 1972). Early work on the metabolism of methylglyoxal focused on its conversion to lactate by glyoxalases and the subsequent oxidation of lactate to pyruvate, thus forming an alternate route to this key intermediate from dihydroxyacetone phosphate.

The methylglyoxal synthase of *E. coli* has been purified (Hopper and Cooper 1972) and characterized. Features reported for the enzyme included a usual pH optimum around 7.5 and a molecular weight of 67 kDa by gel filtration (Hopper and Cooper 1971). Analysis by SDS-gel electrophoresis suggested a mass of 17 kDa for the monomer (Saadat and Harrison 1998). The mechanism of the enzyme has been studied and reviewed (Cooper 1984) and proceeds by the oxidation of the hydroxymethyl group of dihydroxyacetone phosphate to yield the aldehyde of methylglyoxal; and a deprotonation and reduction reaction gives rise to the methyl group. The reaction seems similar to that of triose phosphate isomerase and involves the removal of a C-3 *pro*-S proton followed by β-elimination of the phosphate from the ene–diol enzyme intermediate (Iyengar and Rose 1983). Inhibition and substrate specificity were also analyzed (Iyengar and Rose 1983). The *mgs* gene from *E. coli* has been cloned (Saadat and Harrison 1998; Totemeyer et al. 1998). The sequence of the 152 amino acid *E. coli* methylglyoxal synthase was reported (Percy and Harrison 1996; Totemeyer et al. 1998). Expression of the cloned enzyme has allowed further structural and enzymological studies.

The K_m value of 0.20 mM for dihydroxyacetone phosphate, $k_{cat} = 220\ s^{-1}$, and $k_{cat}/K_m = 1.1 \times 10^6\ M^{-1}\ s^{-1}$ for the isolated recombinant enzyme were similar to those reported earlier for the native enzyme. In the presence of 0.2–0.3 mM phosphate, the kinetics were

Table 1 Methylglyoxal reductase activity of several oxidoreductases

Enzyme	Cofactor	Source	Size	K_m Methylglyoxal	Intermediate product	Related references
Aldehyde dehydrogenase	NADPH	*Escherichia coli*	100 kDa, native	0.77 mM	Acetol	Misra et al. (1996)
Alcohol dehydrogenase	NADH	*E. coli*	100 kDa, native	3.8 mM	Acetol	Misra et al. (1996)
L-Glycol dehydrogenase	NADH	*Enterobacter aerogenes*	30 kDa	75 mM	Lactaldehyde	Carballo et al. (1993)
Methylglyoxal reductase	NADPH	*Saccharomyces cerevisiae*	43 kDa	5.88 mM	Acetol	Murata et al. (1985); Nakamura et al. (1997)
Methylglyoxal reductase	NADH	Goat liver	89 kDa, native; 46 kDa, subunit	0.4 mM	Lactaldehyde	Ray and Ray (1984)
L-Glycol dehydrogenase	NADPH	Hen muscle	30 kDa	0.09 mM	Lactaldehyde	Gonzalez et al. (1983)
Dihydrodiol dehydrogenase	NADPH	Dog liver	39 kDa	0.21 mM		Sato et al. (1994)
Methylglyoxal reductase	NADPH	*Aspergillus niger*	36 kDa	15.4 mM (pH 9.0); 1.43 mM (pH 6.5)		Inoue and Kimura (1995)
Lactaldehyde oxidoreductase	NADH	*Escherichia coli*	4×55 kDa	Millimolar	Acetol	Boronat and Aguilar (1981)
Glycerol dehydrogenase	NADH	*E. coli*	39 kDa		R-Lactaldehyde	Wong and Whitesides (1994); Alteras and Cameron (1999)
Aldose reductase AR	NADPH	Human	36 kDa	0.024 mM (pH 6.0–7.0)	Acetol	Vander Jagt et al. (1992); Cao et al. (1998)
Aldehyde reductase 2 ARL-1	NADPH	Human	35 kDa	0.13 mM (pH 7.0–7.5)	Acetol	Vander Jagt et al. (1992); Cao et al. (1998)
Aldehyde reductase	NADPH	*Sporobolomyces salmonicolor*	35 kDa	3 mM		Kita (1996)

sigmoidal, indicating a Hill coefficient of 2.2–3.4. The enzyme has high thermal stability. For a thorough review of the recent work on the mechanism, the reader is referred to Saadat and Harrison (1998, 1999). The crystal structure found a homohexamer, rather than the tetramer supposed from earlier size measurement (Saadat and Harrison 1999). Enzymological work on site-specific mutants of methylglyoxal synthase from *E. coli* has identified important residues in catalysis (Saadat and Harrison 1998). Asp-71 appears to be a catalytic base in the mechanism, with Asp-20 and Asp 101 implicated in subunit interactions.

Other purified methylglyoxal synthases have been reviewed (Cooper 1984). The methylglyoxal synthase from goat liver (Ray and Ray 1981) exhibited a pH optimum of 7.0 and was inhibited to 10% of its activity in the presence of 40 mM phosphate. The methylglyoxal synthase from *C. acetobutylicum* has been purified and characterized and may be less sensitive to phosphate than the methylglyoxal synthase from other organisms (Huang et al. 1999).

Methylglyoxal reductases

A large number of aldose and ketose reductases have been characterized and some of these have significant activity on methylglyoxal. Certain of those most relevant to possible biotechnological applications are mentioned here and in Table 1. Enzymes in *E. coli* which connect methylglyoxal to acetol have been reported (Misra et al. 1996). NADPH- and NADH-dependent enzymes were found, with the NADPH enzymes having a lower K_m for methylglyoxal (0.77 mM) compared to a K_m of 3.8 mM for the NADH-dependent enzyme. Both enzymes had a pH optimum of 5.8–6.6, with a size of ~100 kDa, based on gel filtration experiments. Enzymes which convert methylglyoxal to lactaldehyde have been reported in *E. coli* (Baldoma and Aguilar 1987), goat (Ray and Ray 1981), and yeast (Murata et al. 1985).

Mammalian aldose reductases have been studied and their relation to diabetic symptoms and complications has been of medical interest. Human aldose reductase uses methylglyoxal as a preferred substrate and produces 95% acetol and 5% lactaldehyde, before a slower formation of s-1,2-propanediol (Vander Jagt et al. 1992). A variant aldoreductase, ARL-1, found in the intestine (Cao et al. 1998) was found to have a three-fold higher k_{cat}. These enzymes use NADPH as the reducing cofactor. Among the large family of aldo–keto reductases, many have been isolated from different tissues, where they may have distinct functions. It has been proposed that those found in the intestine and colon may play a role in the detoxification of cytotoxic aldehydes produced by microbial activity in the gut.

The glycerol dehydrogenase (GDH encoded by *gldA*) of *E. coli* uses NADH as the reducing cofactor and can convert methylglyoxal to R-lactaldehyde (Altaras and Cameron 1999). *gldA* has been cloned (Truniger and Boos

1994) and GDH is active on methylglyoxal (Cameron et al. 1998). The chiral production of 1,2-diols by GDH has been studied (Lee and Whitesides 1986). The glycerol dehydrogenase of *Aspergillus* spp. also has activity on methylglyoxal (18% of that on dihydroxyacetone) and uses NADPH as the reducing cofactor (Schuurink et al. 1990). A number of other enzymes capable of reducing methylglyoxal are listed in Table 1.

Physiological effects of methylglyoxal

Methylglyoxal is a cytotoxic metabolite and its potentially harmful reactions are kept in check in microbes by the removal of this compound through the action of glyoxalase (Cooper 1984). The role of glyoxalase I has been shown, since strains overproducing this enzyme are more resistant to methylglyoxal (MacLean et al. 1998). Mutants lacking glyoxalase I (*gloA*; MacLean et al. 1998) and glutathione-deficient mutants (*gshA*) are quite sensitive to methylglyoxal (Ferguson et al. 1998). Glyoxalase III has been purified and is independent of glutathione for the conversion of methylglyoxal to lactate (Misra et al. 1995). Cells which produce lethal amounts of methylglyoxal under specific growth conditions have been reported (Freedberg et al. 1971; Ackerman et al. 1974). Triose phosphate isomerase mutants growing on gluconate show high methylglyoxal levels during the catabolism of glucose (Cooper and Anderson 1970; Ferguson et al. 1998). A mutant constitutive for gluconate catabolic enzymes forms high levels of the gluconate pathway enzymes; and the addition of gluconate leads to a methylglyoxal concentration of 1.1 mM in the medium (Rekarte et al. 1973).

In mammalian cells, methylglyoxal is also found and is determined to be detrimental to in vivo processes through its reaction with proteins and DNA. It appears that methylglyoxal exists mostly in an adduct form in these cells (Chaplen et al. 1998). Overexpression of an aldehyde reductase can protect PC12 cells from glycation reactions that appear to have a role in diabetic complications (Suzuki et al. 1998).

Metabolic engineering and production considerations

This subject was reviewed recently by Cameron et al. (1998) and both theoretical and practical aspects were considered. Since *E. coli* can be readily manipulated genetically and various strains have been used in the large-scale production of amino acids and other compounds, it is a main platform for the studies conducted so far in this area, although yeast has also been investigated (Hoffman 1999). *E. coli* can be grown either aerobically or anaerobically and the theoretical limits of production under each circumstance have been considered (Cameron et al. 1998). In the calculations reported, the most feasible yield from aerobic organisms where the TCA cycle can provide ATP was calculated to be $\sim$1.42 mol mol^{-1} glucose (0.6 g g^{-1} glucose) considering CO_2 as the primary co-product, while for anaerobic growth the value of 1 mol mol^{-1} was expected, with the concomitant production of an equal amount of acetate.

The concentration of product is an important factor in process economics. One element which can limit the maximal product concentration is the level of inhibition of growth derived from the products of metabolism. In the case of 1,2-propanediol, this compound is less toxic to *E. coli* than 1,3-propanediol; and a growth rate of $\mu = 0.5$ h^{-1} is obtained at a concentration of around 100 g 1,2-propanediol l^{-1} (Cameron et al. 1998). Under anaerobic conditions, acetate would be a co-product and would be inhibitory at a low concentration while, under aerobic conditions, the co-product CO_2 could easily be removed from the culture. So far reports from engineered *E. coli* are well below those achieved in *T. thermosaccharolyticum* (Table 2); however the metabolic engineering efforts are at an early stage for this approach. The productivity in terms of grams per liter per hour has not been strenuously evaluated under production conditions.

The isolation of 1,2-propanediol from the culture broth after fermentation would entail separations like those used for 1,3-propanediol or 2,3-butanediol. Since the boiling points of these diols are high, extraction is a possible alternative. The use of a multieffect evaporator or reverse osmosis to concentrate the product to a level approaching the concentration of propanediol (around 200 g l^{-1}) reached in the chemical route (e.g., after hydration of propylene oxide in water) may be possible (Broekhuis et al. 1994; Martin and Murphy 1994). The subsequent concentration would follow that used in current chemical processing (multieffect evaporators, drying towers). The extractive technologies using intermediary formation of complexes with aldehydes (formaldehyde or acetaldehyde; Broekhuis et al. 1994) or organoboronates (Broekhuis et al. 1996) may also show potential for isolating the product.

Genetic engineering for 1,2-propanediol formation

The selection of the appropriate enzymes for the pathway is of importance. The *E. coli* methylglyoxal synthase is sensitive to phosphate, while that characterized from *C. acetobutylicum* seems less inhibited by phosphate (Huang et al. 1999) and may resemble that from *T. thermosaccharolyticum* (Cameron and Cooney 1986). After the formation of methylglyoxal, this intermediate needs to be rapidly converted to the diol, as methylglyoxal is cytotoxic and has been shown to be very inhibitory to growth.

The reduction of methylglyoxal can be mediated by a variety of reductases. In initial studies with the rat lens aldose reductase (Cameron et al. 1998), it appeared that the initial reduction to acetol was much faster than the subsequent reduction to 1,2-propanediol and little of the

Table 2 Reported yields of 1,2-propanediol from microbial culture

Organism	Plasmid or recombinant genes added	Substrate	Product (on analysis)	Concentration of product (g l^{-1})	References
Clostridium sphenoides		Glucose	R-1,2-Propanediol	2	Tran-Din and Gottschalk (1985)
Thermoanaerobacterium thermosaccharolyticum		Glucose	R-1,2-Propanediol	7.9	Cameron and Cooney (1986)
		Glucose	R-1,2-Propanediol	9.0	Sanchez-Rivera et al. (1987)
		Arabinose	R-1,2-Propanediol	4.29	Cameron et al. (1998)
		Galactose (phosphate-limited)		3.56	Cameron et al. (1998)
E. coli AG1	pSEARx rat lens aldose reductase	Glucose		0.1	Cameron et al. (1998)
E. coli AG1	pNEA10 *E. coli* glycerol dehydrogenase	Glucose	R-1,2-Propanediol	0.15	Cameron et al. (1998)
E. coli AG1	pNEA6 *dhaD*	Glucose	R-1,2-Propanediol	0.34	Cameron et al. (1998)
E. coli AG1	pNEA14 *dhaD*		R-1,2-Propanediol	0.25	Altaras and Cameron (1999)
E. coli AG1	pNEA16 *mgs*		R-1,2-Propanediol	0.26	Altaras and Cameron (1999)
E. coli AG1	pNEA30 *mgs*, glycerol dehydrogenase		R-1,2-Propanediol	0.49	Altaras and Cameron (1999)
E. coli BL21 (DE3)	pGLDH, pMGS2 glycerol dehydrogenase, clostridial *mgs*	Fructose		0.3	Huang et al. (1999)
Saccharomyces cerevisiae	*E. coli mgs*	Glucose	R-1,2-Propanediol	0.24	Hoffman (1999)
S. cerevisiae	*E. coli mgs*, glycerol dehydrogenase	Glucose	R-1,2-Propanediol	0.52	Hoffman (1999)

desired S-isomer was formed. Therefore, overexpression of glycerol dehydrogenase was examined; and this modification gave essentially complete conversion of methylglyoxal to the R-isomer of 1,2-propanediol (Cameron et al. 1998; Altaras and Cameron 1999).

A summary of reported values for the production of 1,2-propanediol from various organisms and engineered strains is given in Table 2. Subsequent to the earlier work with natural clostridial strains, more recent work has focused on engineered *E. coli* strains. However, some yeast strains have given encouraging results. In recombinant *E. coli* and yeast strains, the incorporation of a plasmid encoding both methylglyoxal synthase and glycerol dehydrogenase led to the highest production.

The synthesis of R-1,2-propanediol using acetol as substrate by *S. cerevisiae* has been reported (Manzocchi et al. 1988; Santaniello et al. 1992; Kometani et al. 1993). The production of more than 98% of the R-isomer has been achieved by using ethanol as a co-substrate with acetol (Kometani et al. 1995). *S. cerevisiae* has also been shown to be capable of converting methylglyoxal to both acetol and S-lactaldehyde, which can be further converted to S-1,2-propanediol.

Cameron and co-workers have recently investigated the possibility of producing 1,2-propanediol from glucose by genetic and metabolic engineering of *S. cerevisiae* (Hoffman 1999). By expressing the *E. coli* methylglyoxal synthase gene (*mgs*) in *S. cerevisiae*, they were successful in producing about 0.24 g l^{-1} of 1,2-propanediol from glucose. A further increase in 1,2-propanediol production to approximately 0.52 g l^{-1} from 20 g glucose l^{-1} was achieved by co-expressing the *E. coli gldA* in *S. cerevisiae* along with *mgs*. Deletion of the glycerol synthesis pathway, a competing pathway, was found to increase the 1,2-propanediol yield by about 50%, but did not affect the concentration of 1,2-propanediol achieved.

There are several possible options for improving the reduction of methylglyoxal:

1. Mutagenesis or DNA shuffling of the functional *E. coli* glycerol dehydrogenase to give a more robust enzyme. This could be coupled with an in vivo selection for the removal of methylglyoxal.
2. Overexpression of the native *E. coli* activity which reduces R-lactaldehyde to propanediol (Altaras and Cameron 1999).
3. Testing of other methylglyoxal reductases. These would include acetoin reductase, which has some activity on methylglyoxal (Wardwell 1999), and human aldose reductase variants (Cao et al. 1998).
4. Evaluation of the *C. sphenoides* or goat methylglyoxal reductase could be explored. Since these enzymes produce lactaldehyde which (if it has the correct stereochemistry) could be readily reduced by the *E. coli fucO* gene product, such a combination might provide a suitable route.
5. Enhancement of activity levels of the *fucO* gene product, lactaldehyde oxidoreductase, which has

some activity on methylglyoxal (Baldoma and Aguilar 1987) could potentially allow improved reduction.

Lactaldehyde is converted to L-1,2-propanediol by the NADH-utilizing oxidoreductase encoded by *fucO*. Mutants which constitutively express *fucO* have been isolated (Lu et al. 1998) and *fucO* mutants with increased resistance to metal-catalyzed oxidation have also been characterized (Lu et al. 1998). The *fucO* product, propanediol oxidoreductase, has sequence similarity to the alcohol dehydrogenase II of *Zymomonas mobilis* and the ADH4 of *S. cerevisiae* (Conway and Ingram 1989; Cabiscol et al. 1994). Mutants in *ald* and *fucO* accumulated an inhibitory concentration of lactaldehyde when grown on fucose, suggesting that after reduction the diol exits the cell more easily than lactaldehyde; and this property could thus be important in propanediol production (Zhu and Lin 1989).

The use of a host in which the level of dihydroxyacetone phosphate, the precursor of methylglyoxal, is elevated is also useful to consider. Mutations have been described which affect the lower steps of glycolysis and appear to lead to an increased flow through the methylglyoxal bypass (Irani and Maitra 1977). The effect of overexpressing phosphofructokinase on methylglyoxal formation has recently been considered (Emmerling et al. 1999). Also the removal of the glyoxalase pathway would reduce potential competition with the methylglyoxal reduction pathway and perhaps force the new pathway to be retained. Reduction of ethanol and lactate production would also probably have favorable consequences on propanediol production and would retain a greater proportion of reducing power for this pathway.

Outlook for growth and optimization of production conditions

Bacterial biosynthesis of various chemicals from biomass has been profitable in the case of vitamins and amino acids. Yeasts have been traditional producers of ethanol and provide large-scale quantities for fuel additives. Technological advances for making genetically engineered strains, improvements in measurement of compounds, and theoretical analysis of metabolic fluxes have contributed to the design of production systems for the manufacture of high value-added chemicals. Recent sequencing efforts on a wide variety of microbial and eukaryotic genomes have generated a wealth of information on the genes encoding metabolic enzymes. The analysis of useful genes from biodiverse environments of all kinds will further supply metabolic engineers with sources of new enzymes with useful properties. These efforts and the development of methods to use ''directed evolution'' or ''DNA shuffling'', coupled with powerful high-throughput screening protocols will allow enzymes with optimized performance in the desired pathway to be fashioned. The use of these designed enzyme systems in appropriate genetic constructs affords metabolic engineering of high-flux through the pathway to give a high net yield of product.

The industrial activity on microbial 1,3-propanediol production has also stimulated interest in 1,2-propanediol formation through a biological route. The recent work on genes encoding methylglyoxal synthases and reductases from various organisms and the combination of these genes within *E. coli* to produce a pathway for propanediol production indicate considerable advances lie ahead. The dual future strategies of optimized hosts and optimized expression, together with the enzymatic properties of the pathway enzymes will allow the continued advancement of such processes. The techniques for the removal of competing pathways and the enhancement of desired pathways via genetics and culture manipulation will be important in the development of such a process. The potential for integrating a biological process into the downstream bioprocessing also seems tractable.

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