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# Metabolic engineering of *Clostridium acetobutylicum* for the industrial production of 1,3-propanediol from glycerol

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**Abstract**

*Clostridium butyricum* is to our knowledge the best natural 1,3-propanediol producer from glycerol and the only microorganism identified so far to use a coenzyme $B_{12}$-independent glycerol dehydratase. However, to develop an economical process of 1,3-propanediol production, it would be necessary to improve the strain by a metabolic engineering approach. Unfortunately, no genetic tools are currently available for *C. butyricum* and all our efforts to develop them have been so far unsuccessful. To obtain a better "vitamin $B_{12}$-free" biological process, we developed a metabolic engineering strategy with *Clostridium acetobutylicum*. The 1,3-propanediol pathway from *C. butyricum* was introduced on a plasmid in several mutants of *C. acetobutylicum* altered in product formation. The DG1(pSPD5) recombinant strain was the most efficient strain and was further characterized from a physiological and biotechnological point of view. Chemostat cultures of this strain grown on glucose alone produced only acids (acetate, butyrate and lactate) and a high level of hydrogen. In contrast, when glycerol was metabolized in chemostat culture, 1,3-propanediol became the major product, the specific rate of acid formation decreased and a very low level of hydrogen was observed. In a fed-batch culture, the DG1(pSPD5) strain was able to produce 1,3-propanediol at a higher concentration (1104 mM) and productivity than the natural producer *C. butyricum* VPI 3266. Furthermore, this strain was also successfully used for very long term continuous production of 1,3-propanediol at high volumetric productivity ($3\,g\,L^{-1}\,h^{-1}$) and titer (788 mM).


*Keywords:* 1; 3-propanediol; Glycerol; *Clostridium acetobutylicum*; Metabolic engineering; Vitamin $B_{12}$-free biological process

## 1. Introduction

For a long time, 1,3-propanediol (1,3-PD) has been considered a specialty chemical. However, the recent development of a new polyester called poly(propylene terephtalate), with unique properties for the fiber industry (Miller, 2000; Rudie, 2000), necessitates a drastic increase in the production of this chemical. There are currently two processes for the chemical synthesis of 1,3-propanediol. Both of these processes produce toxic intermediates and require a reduction step under high hydrogen pressure (Sullivan, 1993). The biological production of 1,3-propanediol from glycerol was demonstrated for several bacterial strains, e.g., *Lactobacillus brevis* and *buchnerii* (Schütz and Radler, 1984; Sobolov and Smiley, 1959), *Bacillus welchii* (Humphreys, 1924), *Citrobacter freundii*, *Klebsiella pneumoniae* (Lin and Magasanik, 1960; Ruch et al., 1957; Streekstra et al., 1987), *Clostridium pasteurianum* (Luers et al., 1997) and *Clostridium butyricum* (Biebl et al., 1992; Heyndrickx et al., 1991; Saint-Amans et al.,

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2001). Among those microorganisms, *C. butyricum* is to our knowledge the best "natural producer" both in terms of yield and titer of 1,3-propanediol produced (Saint-Amans et al., 1994). However, to develop an economical process of 1,3-propanediol production, it is necessary to further improve the process by a metabolic engineering approach on the strain. No genetic tools are currently available for *C. butyricum* and all our efforts to develop them have been so far unsuccessful. On the other hand, we recently characterized, from a biochemical (Saint-Amans et al., 2001) and a molecular point of view (Raynaud et al., 2003), the $B_{12}$-independent pathway converting glycerol to 1,3-propanediol in *C. butyricum*. This work opens the possibility to convert other clostridia to 1,3-propanediol producers by the heterologous expression of the genes encoding the $B_{12}$-independent 1,3-propanediol pathway. Among the clostridia, *Clostridium acetobutylicum* is a microorganism of choice as (i) it has already been used for the industrial production of solvent (Cornillot and Soucaille, 1996) and (ii) the genetic tools for gene knockout or gene over-expression are currently available (Mermelstein and Papoutsakis, 1993; Green et al., 1996). The objective of the present work is to develop a recombinant strain of *C. acetobutylicum* for the conversion of glycerol to 1,3-propanediol at higher titer and productivity and if possible higher yield than those obtained in *C. butyricum*. We succeeded for the first two objectives but we failed in the yield improvement due to the metabolic flexibility of *C. acetobutylicum*.

## 2. Material and methods

### 2.1. Bacterial strains and plasmids

All bacterial strains and plasmids used or derived from this study are listed in Table 1.

### 2.2. DNA isolation and manipulation

Plasmid DNA was extracted from *Escherichia coli* with the Qiaprep Kit (Qiagen, Courtaboeuf, France). DNA restriction enzymes, CIP enzyme and $T_4$ DNA ligase were obtained from New England Biolabs (Beverly, Mass) or GIBCO/BRL (Life Technologies, Cergy Pontoise, France) and used according to the manufacturer's instructions. DNA fragments were purified from agarose gels with the QIAquick gel purification kit (Qiagen).

### 2.3. Plasmids and genetic construction

The 5.7 kb pTLH1 plasmid was digested with SalI restriction enzyme and treated with the CIP enzyme. The pSPD5 plasmid was digested with SalI and ClaI restriction enzymes. The 5.1 kb fragment obtained was gel purified and ligated to the SalI linearized pTLH1 vector to yield the 10.9 kb pTLP plasmid.

Prior to the transformation of *C. acetobutylicum* strains (ATCC 824, DG1 or ATCC 824 PJC4BK), pPSD5 and pTLP plasmids were methylated in vivo in *E. coli* ER2275 (pAN1) (Mermelstein and Papoutsakis, 1993), concentrated and desalted on a microcon 100 microconcentrator (Amicon, Inc., Beverly, Mass). Methylated plasmids DNA were used to transform *C. acetobutylicum* by electroporation as previously described (Mermelstein et al., 1992).

### 2.4. Culture media

*E. coli* strains were grown aerobically at 37 °C in Luria–Bertani medium supplemented, when necessary with chloramphenicol (30 μg/ml) and ampicillin (100 μg/ml) or tetracycline (10 μg/ml). The synthetic medium used for clostridia cultivations contained per liter of

Table 1
Bacterial strains and plasmids

| Strain/Plasmid | Relevant characteristics[a] | Source/reference |
|---|---|---|
| *C. acetobutylicum* | | |
| ATCC 824 | Wild type | ATCC, Rockville, MD, USA |
| DG1 | Cured from pSOL1 | Nair (1995) |
| PJC4BK | $BK^-MLS^r$ | Green et al. (1996) |
| *E. coli* | | |
| ER 2275 | $recA^-McrBC^-$ | New England Biolabs |
| *Plasmids* | | |
| pIMP1 | $MLS^r$, $Ap^r$, control plasmid | Mermelstein and Papoutsakis (1993) |
| pAN1 | $Cm^r$, Φ3TI | Mermelstein and Papoutsakis (1993) |
| pSPD5 | $MLS^r$, $Ap^r$, *dhaB1*, *dhaB2*, *dhaT* | Raynaud et al. (2003) |
| pTLH1 | $Tc^r$, $Ap^r$, control plasmid | Harris et al. (2000) |
| pTLP | $Tc^r$, $Ap^r$, *dhaB1*, *dhaB2*, *dhaT* | This study |

[a]Abbreviations: BK: butyrate kinase; $MLS^r$: macrolide, lincosamide, Streptogramin B resistance; $RecA^-$: homologous recombination abolished; $McrBC^-$: lacking methylcytosine-specific restriction system; $Ap^r$: ampicillin resistance; $Cm^r$: chloramphenicol resistance; $Tc^r$ tetracycline resistance; Φ3TI: Φ3T methylase.