Electrochemical control of bacterial metabolism and its application to bioproduction

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Research objective

Currently, various useful chemicals are produced by fermentation using microorganisms. However, there are many chemicals that cannot be produced by conventional fermentation processes at a practical level owing to their low production efficiencies. One reason for the low production efficiencies is that organic substrates, such as sugars, are consumed for the growth and maintenance of microbial cells. The fermentative production of highly reduced chemicals at high yields is particularly difficult as it requires more substrates to generate reducing equivalents (e.g., NADH) and ATP. This can be solved through the use of external energy sources for the bioconversion of substrates; however, such energy sources must be less expensive than organic substrates to maintain the economic feasibility of the entire process.

Electrochemically active bacteria (EAB) have recently gained attention in the field of environmental microbiology[1]. In the presence of high-potential electrodes, these bacteria grow by releasing electrons generated by the oxidative metabolism of organic substrates to the electrodes. However, in the presence of low-potential electrodes, EAB can take up electrons from the electrodes and use them for reductive metabolism. Since electricity is cheaper than sugars (e.g., glucose), its use is expected to reduce the cost of fermentation processes.

Therefore, this study aimed to establish the basis of "electrofermentation" (EF) as a technology that can be widely used to improve the efficiency of the fermentation process. In particular, the present study aimed to develop a process to improve the production yield of 1,4-butanediol (1,4-BDO) by supplying electricity to genetically modified *Shewanella*, an EAB, from low-potential electrodes.

Methods

Shewanella oneidensis MR-1 and its derivatives were cultured at 30 °C in LB or minimal medium[2] containing lactate or glucose as the sole carbon and energy source. Transformation and gene disruption were carried out by filter mating and double-crossover methods, respectively[2]. For transcriptome analysis using DNA microarrays, MR-1(pBBR-*glk-galP*) strains were precultured in glucose-minimal medium under fumarate-reducing conditions. The cells were then cultured under fermentation (in the

absence of electron acceptors) or fumarate-reducing conditions for 3 h, after which total RNA was extracted. DNA microarray analysis was performed as described previously[3].

Results

1. Evaluation of the glucose fermentation ability of genetically modified *Shewanella*

S. oneidensis MR-1 is a promising host for EF due to its ability to receive electrons from low-potential electrodes and use them for intracellular reductive metabolism[1]. Although wild-type MR-1 cannot utilize sugars (glucose), a previous study has shown that an engineered strain of MR-1 (MR-1[pBBR-*glk-galP*]) expressing the genes responsible for glucose uptake and phosphorylation (*galP* and *glk*, respectively) can utilize glucose under fumarate-reducing conditions[2]. However, it remains unclear whether this mutant strain could grow on glucose under fermentation conditions. Therefore, we first inoculated MR-1(pBBR-*glk-galP*) into glucose minimal medium and examined whether it was able to grow under fermentation conditions. The results revealed that this strain cannot grow on glucose minimal medium under fermentation conditions but can grow in the presence of electron acceptors, such as oxygen and fumarate. The inability to grow under fermentation conditions may be attributed to decreased activity of the TCA cycle and amino acid synthesis because MR-1 is known to repress the TCA cycle activity under reductive conditions. This hypothesis was supported by the results of the comparative transcriptome analysis of MR-1 grown under fermentative and fumarate-reducing conditions. We cultivated MR-1(pBBR-*glk-galP*) in glucose minimal medium in the presence of 0.1% (w/v) tryptone as the amino acid source and found that supplementation with tryptone enabled the fermentative growth of this strain on glucose. This finding suggests that the MR-1(pBBR-*glk-galP*) strain can be used as a host for EF using glucose as a substrate if a small amount of an amino acid source is supplied.

2. Introduction of 1,4-BDO synthesis genes and disruption of byproduct synthesis genes

To confer the ability to synthesize 1,4-BDO on MR-1(pBBR-*glk-galP*), we introduced the genes required for 1,4-BDO synthesis (Fig. 1)[4] into this strain and disrupted the pathways involved in the production of byproducts (i.e., lactate and acetate). In addition, we introduced the pyruvate decarboxylase gene (*pyc*) derived from *Bacillus subtilis* into MR-1(pBBR-*glk-galP*) to facilitate the conversion of glucose to succinate, a precursor of 1,4-BDO. Currently, we are confirming the expression of the genes shown in Fig. 1 and the activity of each enzyme.

adhE2: Aldehyde-alcohol dehydrogenase from *Clostridium acetobutylicum*

cat2: 4-Hydroxybutyryl-CoA transferase from *Porphyromonas gingivalis*

sucCD : Succinyl-CoA synthetase from *Escherichia coli* K-12

sucD: Semialdehyde dehydrogenase from *P. gingivalis*

4HBd: 4-Hydroxybutyrate dehydrogenase from *P.gingivalis*

Fig. 1. Gene cassette for 1,4-BDO synthesis

Conclusion

Here, we showed that an engineered strain of MR-1 expressing the genes for glucose uptake and phosphorylation (*galP* and *glk*, respectively), MR-1(pBBR-*glk-galP*), is capable of fermentatively metabolizing glucose and is suitable as a host for EF. In addition, a gene cassette for the synthesis of 1,4-BDO was designed and introduced into MR-1(pBBR-*glk-galP*). In the future, we plan to examine whether an MR-1(pBBR-*glk-galP*)-based engineered strain can synthesize 1,4-BDO by EF in the presence of a low-potential electrode (-0.6 V vs. Ag/AgCl).

References

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