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Combining metabolic engineering and adaptive evolution to enhance the production of dihydroxyacetone from glycerol by *Gluconobacter oxydans* in a low-cost way

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ABSTRACT

Gluconobacter oxydans can rapidly and effectively transform glycerol to dihydroxyacetone (DHA) by membrane-bound quinoprotein sorbitol dehydrogenase (mSLDH). Two mutant strains of GDHE Δ*adh* pBBR-P_{tufB}*sldAB* and GDHE Δ*adh* pBBR-*sldAB* derived from the GDHE strain were constructed for the enhancement of DHA production. Growth performances of both strains were largely improved after adaptively growing in the medium with glucose as the sole carbon source. The resulting GAT and GAN strains exhibited better catalytic property than the GDHE strain in the presence of a high concentration of glycerol. All strains of GDHE, GAT and GAN cultivated on glucose showed enhanced catalytic capacity than those grown on sorbitol, indicating a favorable prospect of using glucose as carbon source to reduce the cost in industrial production. It was also the first time to reveal that the expression level of the *sldAB* gene in glucose-growing strains were higher than that of the strains cultivated on sorbitol.

1. Introduction

Dihydroxyacetone (DHA) is one of important chemical and biochemical materials, which is used as sunless tanning agent, pharmaceutical precursors and functional additives (Claret et al., 1994; Hekmat et al., 2003). The most popular industrial form of producing DHA is the biotransformation of glycerol to DHA with *Acetobacter* species, *Gluconobacter* species and *Yeast* species, especially *Gluconobacter oxydans*. The microbial processes were preferred because of their specific nature and the mild process environment. In the past, great efforts had been devoted to the screening of excellent strains, optimization of medium composition and fermentation parameters (Bauer et al., 2005; Fidaleo et al., 2006; Hekmat et al., 2003; Wei et al., 2007, 2009; Wethmar and Deckwer, 1999). Recent advances in genetic manipulation have allowed the elimination of product or substrate inhibition and the improvement of DHA production. It was reported that the overexpression of the *sldAB* gene that encodes membrane-bound sorbitol dehydrogenase (mSLDH) could obviously enhance the production of DHA (Gatgens et al., 2007; Li et al., 2010). Recent evidence has also shown that the growth of *G. oxydans* in a high concentration of glycerol was largely improved when the membrane-bound alcohol dehydrogenase (ADH) (encoded by the *adh* gene) was deficient (Habe et al., 2009a, 2009b, 2010; Li et al., 2010).

Glucose is the favorable carbon source for many microorganisms, but not for *G. oxydans* which prefers to use glycerol, sorbitol or mannitol. When glucose is used as carbon source, only a minor part of glucose is assimilated in the cytoplasm by the $NADP^+$-dependent soluble glucose dehydrogenase (sGDH) and further dissimilated via the pentose phosphate (PP) pathway or the Entner-Doudoroff (ED) pathway (Holscher et al., 2009; Olijve and Kok, 1979; Rauch et al., 2010). The majority of glucose is directly oxidized to gluconate by membrane-bound pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (mGDH, encoded by the *mgdh* gene) in the periplasm space. The obtained gluconate is further oxidized to 2-ketogluconate, 2,5-diketogluconate as well as 5-ketogluconate, which are then secreted almost completely into the medium, causing extremely acidic environment and therefore significant inhibition of cell growth (Gupta et al., 2001; Holscher and Gorisch, 2006; Holscher et al., 2009). In addition, the dissolved oxygen was mainly consumed for the oxidation process of glucose by mGDH, resulting in severe oxygen limitation on the growth of the wild-type strain in glucose medium (Silberbach et al., 2003).

In industrial production, it is preferable to obtain large amount of *G. oxydans* cells as biocatalyst on relatively cheap carbon source. It was indicated that mutants deficient in mGDH could exhibit

Abbreviations: ADH, membrane-bound alcohol dehydrogenase; C_{max}, maximum cell density; DHA, dihydroxyacetone; HPLC, high performance liquid chromatography; mGDH, pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase; mSLDH, membrane-bound quinoprotein sorbitol dehydrogenase; PCR, polymerase chain reaction; PQQ, pyrroloquinoline quinine; qRT-PCR, quantitative real-time PCR; RT-PCR, reverse transcription PCR; sGDH, $NADP^+$-dependent soluble glucose dehydrogenase; μ_{max}, maximum specific growth rate.

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