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Methanol-Essential Growth of *Corynebacterium glutamicum*: Adaptive Laboratory Evolution Overcomes Limitation due to Methanethiol Assimilation Pathway

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Abstract: Methanol is a sustainable substrate for biotechnology. In addition to natural methylotrophs, metabolic engineering has gained attention for transfer of methylotrophy. Here, we engineered *Corynebacterium glutamicum* for methanol-dependent growth with a sugar co-substrate. Heterologous expression of genes for methanol dehydrogenase from *Bacillus methanolicus* and of ribulose monophosphate pathway genes for hexulose phosphate synthase and isomerase from *Bacillus subtilis* enabled methanol-dependent growth of mutants carrying one of two independent metabolic cut-offs, i.e., either lacking ribose-5-phosphate isomerase or ribulose-5-phosphate epimerase. Whole genome sequencing of strains selected by adaptive laboratory evolution (ALE) for faster methanol-dependent growth was performed. Subsequently, three mutations were identified that caused improved methanol-dependent growth by (1) increased plasmid copy numbers, (2) enhanced riboflavin supply and (3) reduced formation of the methionine-analogue O-methyl-homoserine in the methanethiol pathway. Our findings serve as a foundation for the engineering of *C. glutamicum* to unleash the full potential of methanol as a carbon source in biotechnological processes.

Keywords: synthetic methylotrophy; methanol; ribulose monophosphate pathway; adaptive laboratory evolution; isotopic labeling; metabolic engineering

1. Introduction

Long-term transition from fossil fuels to renewable energy is inevitable and poses one of the major challenges for humanity in the 21st century. Methanol is a promising alternative renewable energy source with a price of 359 USD per metric ton (April 2020, Methanex, https://www.methanex.com/our-business/pricing) and the feasibility of a "methanol economy" has been proposed before [1]. Methanol can be synthesized from gasified biomass wastes or environmentally captured CO_2—electrochemically or by hydrogenation. Therefore, pure H_2 is required which can be gained from electrolysis of water, powered by solar, wind or thermal energy. [2]. Moreover, the conversion of CO_2 to methanol by